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THE UNIVERSITY OF ALBERTA

THE NEURONAL CONTROL OF JUMPING IN THE LOCUST

by

IAN C. GYNTHER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA

FALL 1988

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ABSTRACT

The neuronal mechanisms underlying jumping in the locust were investigated using a preparation which enabled intracellular recording from thoracic neurons during bilateral kicks (a behavior with a motor program similar to that for a jump). The main aim of the study was to test the existing hypotheses that : a) the jump is triggered by the activity of a pair of identified metathoracic interneurons, the M-neurons, which inhibit hindleg flexor motoneurons, b) the excitability of the M-neurons is increased by proprioceptive feedback from the hindleg during the program's co-contraction phase, and c) the M-neurons eventually discharge when the proprioceptive input depolarizes them beyond threshold, or when there is additional input from exteroceptive stimuli.

In support of the M-neuron's proposed involvement in the triggering process, these neurons were found to discharge powerfully at the time flexor activity was terminated. Also, injecting strong depolarizing current pulses into an M-neuron could trigger extension of one leg. However, suppressing the M-neuron's discharge did not prevent kicks from being triggered. The M-neurons cannot, therefore, be solely responsible for terminating flexor activity. An additional pair of interneurons was identified which may function in this role.

Contrary to the hypothesis that the M-neurons are gradually depolarized by proprioceptive input during co-contraction, they were found to be hyperpolarized at this time. Furthermore, the proposal that exteroceptive stimuli are able to trigger jumps was not upheld because these stimuli failed to elicit kicks or to evoke discharges in the M-neurons during co-contraction. The evidence indicates that trigger activity in the M-neurons is produced by excitatory input from a central interneuronal system. The neurons which make up this trigger system were not identified, but many possible candidates have been found.

Kicking and jumping are known to be dependent on feedback from hindleg proprioceptors. To determine which receptors might be important, afferent recording, stimulation and ablation experiments were performed. The main finding was that tonic feedback from the femoral chordotonal organ was required for both the co-contraction and trigger phases to occur. The way in which chordotonal input is integrated by the central

circuits to generate the motor program for kicks and jumps remains to be determined.

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Table of Contents

Chapter — Page

I. INTRODUCTION 1

A. CELLULAR MECHANISMS UNDERLYING BEHAVIOR 1

Central Pattern Generation 2

Initiation of Behaviors by Single Neurons 3

The Role of Sensory Input in Controlling Motor Activity 4

Chemical Modulation of Neural Circuits 6

B. CELLULAR ANALYSIS OF BEHAVIOR IN INSECTS 7

Interneurons in the Thoracic Ganglia 7

Circuitry Underlying Behavior 10

C. REFERENCES 14

II. INTRACELLULAR RECORDINGS FROM INTERNEURONS AND MOTONEURONS DURING BILATERAL KICKS : IMPLICATIONS FOR MECHANISMS CONTROLLING THE JUMP 23

A. INTRODUCTION 23

B. MATERIALS AND METHODS 24

EMG Recording from Intact Animals 25

Preparation of Animals 25

Intracellular Recording and Dye-filling 26

C. RESULTS 26

Motor Patterns During Jumps and Bilateral Kicks 26

Recordings from Motoneurons During Bilateral Kicks 30

Recordings from Interneurons During Bilateral Kicks 35

D. DISCUSSION 48

The Motor Program 48

Triggering of the Jump 51

E. REFERENCES 53

III. AN EVALUATION OF THE ROLE OF IDENTIFIED INTERNEURONS IN TRIGGERING KICKS AND JUMPS 55

A. INTRODUCTION 55

B. MATERIALS AND METHODS 58

Preparation of Animals 58

Intracellular Recording and Dye-filling 58

Timing of Pulses During Co-contraction 60

C. RESULTS 61

Are the M-neurons Sufficient to Trigger Kicks? 64

Are the M-neurons Necessary for Triggering Kicks? 74

A Parallel Pathway for Terminating Flexor Activity 79

D. DISCUSSION 82

Organization of the Jump Trigger System 82

Role of the M-neurons in Triggering Kicks and Jumps 87

Comparison With Other Systems 90

E. REFERENCES 91

IV. EVIDENCE THAT EXTEROCEPTIVE INPUT DOES NOT TRIGGER KICKS AND JUMPS 94

A. INTRODUCTION 94

B. MATERIALS AND METHODS 96

Preparation of Animals 96

Intracellular Recording and Dye-filling 96

Presentation of Sensory Stimuli 97

C. RESULTS 98

D. DISCUSSION 107

Hyperpolarization or Depolarization? A Conflict Resolved 107

Exteroceptive Inputs Do Not Trigger Kicks 109

E. REFERENCES 112

V. THE ROLE OF PROPRIOCEPTIVE INPUT IN THE JUMP SYSTEM 114
A. INTRODUCTION 114
B. MATERIALS AND METHODS 116
Nerve Recording and Stimulation 116
Nerve and Receptor Apodeme Transection 117
C. RESULTS 118
The Role of the Lump Receptor 118
The Role of the Chordotonal Organ 126
D. DISCUSSION 141
Lump Receptor Input Does Not Trigger Kicks and Jumps 141
Contribution of the Chordotonal Organ to the Motor Program 143
E. REFERENCES 149
VI. COUPLING OF FLIGHT INITIATION TO THE JUMP 150
A. INTRODUCTION 150
B. MATERIALS AND METHODS 151
C. RESULTS 152
EMG Recordings From Flight and Leg Muscles 152
Intracellular Recordings From Flight Motoneurons and Interneurons 157
D. DISCUSSION 165
E. REFERENCES 171
VII. SUMMARY AND CONCLUSIONS 173
A. ORGANIZATION OF THE JUMP SYSTEM 173
B. FUTURE WORK 179
C. REFERENCES 181

LIST OF TABLES

Table		Page
6.1.	Correlation between the initial response to wind on the head and the response during co-contraction in identified flight interneurons	163

LIST OF FIGURES

Figure		Page
1.1.	Diagram of the femur and proximal tibia of the hindleg	1
2.1.	Electromyographic recordings from hindleg flexor and extensor tibiae muscles during jumps and bilateral kicks	28
2.2.	Intracellular recordings from the fast extensor tibiae motoneuron during kicks	31
2.3.	Intracellular recordings from flexor tibiae motoneurons during kicks	34
2.4.	Intracellular recordings from the M-neuron during kicks	37
2.5.	Intracellular recording from an M-neuron made while the ipsilateral leg was prevented from kicking	40
2.6.	Intracellular recording from the C-neuron during a kick	42
2.7.	Intracellular recordings from two mesothoracic interneurons during kicks	44
2.8.	Intracellular recordings from two metathoracic interneurons during kicks	46
2.9.	Intracellular recording during kicking from a local interneuron in the metathoracic ganglion	47
3.1.	Preparation used to study bilateral kicking, and a typical example of the motor program for this behavior	59
3.2.	Precise correspondence between the timing of the M-neuron's discharge and the onset of flexor repolarization during a bilateral kick	62
3.3.	Flexor repolarization commencing before the discharge in the M-neuron	63
3.4.	Timing of the M-neuron's discharge relative to the start of tibial extension during a bilateral kick	65
3.5.	Premature triggering of leg extension produced by strongly depolarizing an M-neuron	66
3.6.	Premature triggering of one leg does not affect the time of extension of the opposite leg	68
3.7.	Lack of direct coupling between the two M-neurons during a bilateral kick	69
3.8.	Incomplete flexor repolarization during a kick evoked by a depolarizing pulse in M	72

3.9.	Differences in the nature of flexor repolarization during natural and pulse-evoked kicks	73
3.10.	Depolarizing current pulses in an M-neuron hyperpolarize ipsilateral flexor motoneurons	75
3.11.	Abolishing spike activity in an M-neuron does not prevent bilateral kicking	76
3.12.	The rate of flexor repolarization during a kick is slowed when M-neuron activity is suppressed	78
3.13.	Another pair of interneurons involved in triggering kicks	80
3.14.	The discharge in 707 occurs at the correct phase for it to contribute to the termination of flexor activity	81
3.15.	Pulse-evoked activity in 707 can trigger leg extension during a bilateral kick	83
4.1.	Medial and lateral processes of the M-neuron can receive different synaptic inputs during bilateral kicks	99
4.2.	Variations in the pattern of synaptic activity in the M-neuron's lateral branch during kicks	100
4.3.	The effect of visual stimulation on the M-neuron in the quiescent animal and during co-contraction	102
4.4.	The effect of auditory stimulation on the M-neuron in the quiescent animal and during co-contraction	104
4.5.	Response of the G-neuron to auditory stimulation in the quiescent animal and during co-contraction	106
5.1.	Transection of the lateral nerve has no effect on the kick motor program	119
5.2.	Afferent activity recorded from the lateral nerve during a kick	120
5.3.	Premature repolarization produced by lateral nerve stimulation during co-contraction	122
5.4.	Lateral nerve stimulation causing premature leg extension during co-contraction does not activate the ipsilateral M-neuron	124
5.5.	Effects of lateral nerve stimulation on ipsilateral flexor motoneurons and M-neurons in the quiescent animal	125
5.6.	Severing the receptor apodeme of the chordotonal organ prevents the initiation of the co-contraction phase in the ipsilateral leg	127
5.7.	Chordotonal afferent activity during kicks	130

5.8.	Premature repolarization of ipsilateral flexors produced by chordotonal stimulation during co-contraction	131
5.9.	Chordotonal stimulation causes the termination of the co-contraction phase in the contralateral leg	133
5.10.	Chordotonal stimulation during co-contraction causes the delayed repolarization of contralateral flexor motoneurons	136
5.11.	Chordotonal stimulation during co-contraction activates the ipsilateral M-neuron	137
5.12.	Chordotonal stimulation during co-contraction activates the contralateral M-neuron	138
5.13.	Chordotonal stimulation does not evoke trigger activity or strongly activate the M-neurons when co-contraction has not been initiated	140
6.1.	Kicking in both hindlegs of a restrained locust evokes a short sequence of flight activity	153
6.2.	Progressive increase in the excitation of the flight system during the co-contraction phase of a kick	155
6.3.	Histogram showing the distribution of the interval between the time a kick is triggered and the onset of flight activity	156
6.4.	Intracellular recordings from wing elevator motoneurons during kicks of the hindlegs	158
6.5.	Intracellular recordings from elevator motoneurons during kicks when rhythmic flight activity is not initiated	161
6.6.	Intracellular recordings from depressor motoneurons during kicks	162
6.7.	Intracellular recordings from identified interneurons during a kick and when wind is blown on the head	164
6.8.	Intracellular recordings from two flight interneurons showing a strong depolarizing response during the co-contraction phase of a kick	166
6.9.	Schematic diagram summarizing the main features of the coupling between the 'jump system' and the 'flight system'	170
7.1.	Neuronal organization of the jump system	174

LIST OF ABBREVIATIONS

CNS	central nervous system
CO	chordotonal organ
CPG	central pattern generator
DCMD	descending contralateral movement detector interneuron
EMG	electromyogram
EPSP	excitatory postsynaptic potential
FETi	fast extensor tibiae motoneuron
IPSP	inhibitory postsynaptic potential

I. INTRODUCTION

A. CELLULAR MECHANISMS UNDERLYING BEHAVIOR

The realization that individual nerve cells can have unique identities has contributed greatly to the present level of understanding of the cellular mechanisms underlying behavior. Wiersma (1952), in his studies of the crayfish nerve cord, was the first to demonstrate that single neurons could be reliably identified from animal to animal, and that specific functions could be ascribed to these cells. Since that time, and particularly in the last 15 years, there has been a rapid increase in the catalogued number of identified neurons (e.g. Hoyle 1977), due mainly to improvements in techniques for intracellular recording and cell staining. Although identified neurons are also known in vertebrates (notable examples being the Mauthner cells in the brainstem of fish and amphibians, Faber and Korn 1978), most knowledge of the role of single cells in determining behavior has come from studies of invertebrates. The advantages of invertebrate preparations are numerous. Apart from obvious factors such as availability and low cost, invertebrates on the whole possess simple nervous systems containing relatively few nerve cells which commonly are organized into discrete ganglia. The number of neurons in these ganglia can be very low. For example, there are only nine cells in the cardiac ganglion of crabs and lobsters (Watanabe 1958; Tazaki and Cooke 1979). This, combined with the often large size of the neuronal cell bodies, facilitates the identification of single neurons and enables their penetration with microelectrodes. In addition, intracellular recordings can be made from more than one cell at a time and thus details of synaptic interactions and connectivity can be determined. Perhaps the greatest appeal of invertebrate preparations though is that despite the relative simplicity of their nervous systems, they display a wide variety of behaviors. Moreover, these behaviors often continue to be expressed after the animal has been radically dissected, or its nervous system competely isolated.

One field of interest in analyzing the cellular basis of behavior has been to determine mechanisms by which patterned motor activity is generated, and by which various behaviors

are initiated and subsequently modulated. Studies in these areas have yielded findings of widespread significance, and indeed some have been established as general concepts applicable even to higher mammals. Here the major findings arising from studies of identified invertebrate neurons will be briefly considered.

Central Pattern Generation

In many animals the basic features of rhythmic movements, such as those involved in locomotion, mastication and respiration, are produced by central neuronal systems termed central pattern generators (CPGs, Delcomyn 1980). These are ensembles of neurons which are capable of generating the correct temporal sequence and phasing of motor activity in the complete absence of sensory feedback. Considerable progress has been made towards elucidating the cellular organization and functioning of CPGs in invertebrates (Kristan 1980; Selverston and Moulin 1985; Getting 1986) and this has revealed a wide diversity of mechanisms for producing patterned motor output. The normal pattern may arise as an emergent property of the network of neurons, or its generation may depend on intrinsic properties of neurons within the oscillator. Thus, for example, the pattern and nature of synaptic connectivity between interneurons is mainly responsible for producing the swimming rhythm in *Tritonia* (Getting 1981, 1893a,b; Getting and Dekin 1985). Complex synaptic interactions (multicomponent PSPs) between groups of cells, giving rise to circuits of reciprocal inhibition paralleled by delayed excitation, are the basis of the CPG in this system. The importance of intrinsic cellular properties for pattern generation is highlighted in the lobster stomatogastric ganglion. Here, membrane characteristics of individual neurons, including postinhibitory rebound properties and ability to generate bursting pacemaker potentials and plateau potentials, make important contributions to the generation of the pyloric rhythm (Miller and Selverston 1982, 1985). However, it would not be correct to suggest that the functioning of CPGs depends solely on the oscillatory properties of either individual neurons or of the network as a whole because elements of both pattern generating

mechanisms are incorporated into most systems (e.g. Getting 1983c; Selverston et al. 1983).

A notable feature of all CPG networks that have been examined to date is an unexpected degree of complexity in their circuitry. An extreme example is in *Tritonia* where 79 out of 132 possible monosynaptic connections between the 12 neurons of the swim network have been found to exist (Getting 1986). One consequence of this complexity is that there is often a certain amount of redundancy in the underlying oscillatory mechanisms such that components of the network can be removed, or circuits disrupted, without abolishing rhythmicity (e.g. Miller and Selverston 1982; Getting 1983b; Robertson and Pearson 1985). Also, the wide diversity in circuitry in the different systems makes it difficult to generalize about the organization of CPGs. However, the various circuits do appear to be constructed from a common set of component mechanisms, so that the organizational differences between oscillatory systems is simply a reflection of the particular way in which these basic components are combined (Getting 1986).

Initiation of Behaviors by Single Neurons

Individual identified interneurons, or groups of such neurons, have been found to play important roles in the initiation of behavior. In most known examples these neurons are involved in initiating rhythmic movements by activating particular CPGs. This has been demonstrated in leeches (e.g. Weeks and Kristan 1978; Weeks 1982; Brodfuehrer and Friesen 1986; Nusbaum 1986), molluscs (e.g. Granzow and Kater 1977; Rose and Benjamin 1981; Fredman and Jahan-Parwar 1983; McCrohan 1984), crustaceans (Davis and Kennedy 1972a,b) and insects (Ritzmann et al. 1980; Bicker and Pearson 1983; Pearson et al. 1985; Boyan et al. 1986). However, individual neurons can also elicit behavioral responses involving brief, non-rhythmic patterns of motor activity. Often these can be classified as startle or escape responses. Classical examples are the C-type fast starts elicited by Mauthner neurons in teleost fish and amphibian larvae (reviewed by Eaton and Hackett 1984) and the short latency tailflips in crayfish produced by spikes in either the medial or lateral giant fibers (reviewed by

Wine and Krasne 1982).

The existence of identified neurons which can initiate behaviors led to the formulation of the command neuron concept (Wiersma and Ikeda 1964; reviewed by Kupfermann and Weiss 1978). According to this idea, a particular behavior is switched on by an individual command neuron or a discrete group of neurons (command system). In order to be designated as having such a command function, it was proposed that a neuron should be both necessary and sufficient for the initiation of the behavior and that it be active during the normal execution of that behavior (Kupfermann and Weiss 1978). While many neurons in the various motor systems meet the sufficiency criterion, few have also been found to be necessary for evoking the behavioral response (e.g. Olson and Krasne 1981; Fredman and Jahan-Parwar 1983). Moreover, in operational terms it has often been difficult to define individual cells as being command neurons because in some instances these neurons receive excitation from the CPG they activate (Gillette et al. 1978) or may actually be members of the CPG network itself (Weeks and Kristan 1978; Davis and Kovac 1981). Nevertheless, the concept has proven useful in that it has focused much attention on the functional organization of neuronal systems underlying behavior and on the role of individual neurons within these systems.

The Role of Sensory Input in Controlling Motor Activity

Although CPGs can produce rhythmic movements without phasic sensory feedback, it has long been recognized that this feedback is necessary to fine-tune the centrally programmed movements and to compensate for environmentally produced perturbations (e.g. Delcomyn 1985). However, a growing number of studies indicate that the influence exerted by sensory input on central neuronal networks extends beyond this. For instance, in the flight system of the locust wing proprioceptors appear to be intimately involved in generating the normal pattern of rhythmic activity (Pearson et al. 1983; Möhl 1985a,b; Wolf and Pearson 1987). Indeed, phasic input from the wing stretch receptors is able to modify the central flight circuit by abolishing activity in some neurons and altering the phase at which other neurons discharge

(Reye and Pearson 1987). As another example, rhythmically stretching a muscle receptor organ at the base of the crayfish walking leg can entrain the central walking rhythm produced by the thoracic ganglia (Sillar et al. 1986). One of the receptor organ's two sensory neurons triggers leg remotion and excites remotor motoneurons during the stance phase; the other triggers leg promotion and initiates the swing phase. A similar situation exists in the walking system of the stick insect where feedback from the femoral chordotonal organ during the stance phase initially excites tibial flexor motoneurons, but later, towards the end of the phase, inhibits these motoneurons and excites the extensors to produce the swing phase (Bässler 1988). The major difference between this system and that of the crayfish is that there is no evidence that walking is produced by a CPG in the stick insect. Nevertheless, in the walking systems of both animals sensory input has at least two important functions : a) to reinforce ongoing motor activity, and b) to cause a switch from one phase of motor activity to another. In the crayfish, the deafferented thoracic ganglia can produce a rhythmic pattern of motor output, but this does not appear to represent a specific behavior in the intact animal (Sillar et al. 1987). These workers concluded that the CPGs of the walking legs are only loosely organized to produce a basic reciprocity, but under the influence of sensory feedback are reorganized to produce an adaptive motor pattern.

In addition to being important for patterning motor output during a given behavior, sensory input can direct the CNS to produce motor patterns for entirely different behaviors, such as different modes of locomotion. In the cockroach, presence or absence of sensory feedback signalling leg contact with the substrate determines whether walking or flight will be produced in response to a cercal wind puff (Ritzmann et al. 1980). Similarly, swimming in the crab is inhibited, and walking facilitated, by feedback from contact-sensitive mechanoreceptors in the leg (Bévengut et al. 1986). In *Tritonia*, sensory inputs of different strengths are able to switch the neural circuitry of the swim oscillator into various configurations. Thus weak stimuli produce only reflexive withdrawals from the animal, but stronger stimuli reorganize the central network to produce swimming behavior (Getting and

Dekin 1985).

These few examples illustrate the major contribution that sensory input can make to the patterning of motor activity in invertebrates. Perhaps, in time, this importance will be revealed to be a general phenomenon.

Chemical Modulation of Neural Circuits

Neurotransmitters, neuromodulators and neurohormones can also act on central networks of neurons to alter the patterns of motor activity they produce. These actions cover a broad spectrum from very general to highly specific modulatory effects. Thus, for instance, the blood-borne serotonin released from identified neurons in the leech serves to prolong the duration of swimming activity, increase the frequency at which bouts of swimming occur and decrease the response time between stimulus presentation and the onset of swimming (Willard 1981; Kristan and Nusbaum 1983; Lent and Dickinson 1984). These effects are just part of the way in which serotonin orchestrates the entire feeding behavior of the leech (Lent and Dickinson 1984, 1988). Aminergic cells in the cerebral ganglia of gastropod molluscs potentiate feeding motor output from the buccal ganglia by modulating the intensity and duration, and in some species also the rate, of feeding movements (Berry and Pentreath 1976; Gillette and Davis 1977; Weiss and Kupfermann 1978). Amines released at central synapses in the crayfish exert specific modulatory actions on transmission in the pathway from sensory afferents to the lateral giant fibers and are thereby able to set the level of excitability of the escape response (Glanzman and Krasne 1983). Another example of a specific effect is in the moth *Manduca* in which a circulating peptide hormone is responsible for activating the neuronal circuitry for a defensive reflex at a particular stage of the insect's development (Levine and Truman 1983).

Central networks can also be tuned by the actions of neurotransmitters in much the same way as they are by sensory inputs. A number of neural inputs to the lobster and crab stomatogastric ganglion release various amine and peptide neurotransmitters which influence

the pyloric pattern generator (reviewed by Marder 1984; Marder et al. 1987). These inputs alter both the voltage-dependent membrane properties of oscillator neurons and the strength and functional importance of synaptic interactions between neurons in the network. The consequence of this is that even simple neuronal networks can produce a variety of patterns of motor activity and thereby may participate in several different behaviors. This, too, may prove to be a finding of general significance.

B. CELLULAR ANALYSIS OF BEHAVIOR IN INSECTS

Insects have proven to be particularly valuable for electrophysiological analyses of the cellular mechanisms underlying behavior. They possess a relatively complex and highly ordered nervous system containing on the order of 10^5 nerve cells. Individual ganglia in the nerve cord may have as many as several thousand neurons. This complexity endows insects with a rich behavioral repertoire and yet does not preclude an examination of the cellular organization of their nervous systems. Recent work on motor systems in insects has concentrated on determining the mechanisms which underlie behaviors such as walking, posture, flight and respiration. These behaviors are all coordinated by the thoracic ganglia and are therefore amenable to intracellular investigation since the thoracic CNS is readily accessible and will continue to produce motor output after dissection. Recordings can be made from the interneurons involved in each behavior and the connections between different neurons can be determined. Here, I shall briefly review the categories and functions of these various thoracic interneurons, before considering what is known about neuronal circuitry for motor behaviors in insects.

Interneurons in the Thoracic Ganglia

Nonspiking Interneurons

Nonspiking interneurons, first discovered in insects by Pearson and Fourtner (1975), are abundant in the CNS of these animals. For example, they are thought to

comprise more than half of the total neuronal complement in the thoracic ganglia of locusts. As their name implies, nonspiking interneurons do not generate action potentials or possess axonal processes. Instead they communicate with postsynaptic cells by graded release of transmitter in response to tonic changes in membrane potential (reviewed by Pearson 1976, 1978; Siegler and Burrows 1980; Burrows 1981; Wilson and Phillips 1983). In such graded interactions, the relationship between the pre- and postsynaptic voltage is commonly of high gain and quite linear (Burrows 1980), so that there is a continuous relationship between input and output (Pearson 1976). This provides high sensitivity and accuracy and ensures that there is minimal loss of information. Also, since under resting conditions nonspiking cells often release transmitter tonically, it is possible for them to signal slight changes in membrane potential in both depolarizing and hyperpolarizing directions. This has led to the idea that much of the processing performed by nonspiking interneurons may take place locally within discrete regions of the neuron's dendritic field. The important consequence of this is that individual interneurons may not act as single functional units in the circuits in which they participate.

Nonspiking neurons have been shown to possess a wide sphere of influence, i.e. single neurons can exert actions (sometimes of opposite sign) on many different motoneurons and are thereby able to call forth coordinated patterns of motor activity (Burrows 1980). This makes them particularly suitable for the regulation of posture (Siegler 1981), although they are by no means limited to such roles. Nonspiking interneurons have, for example, been found to be important in the rhythm generating system underlying walking in the cockroach (Pearson and Fourtner 1975).

Spiking Local Interneurons

Spiking local interneurons are those neurons which generate action potentials, but whose processes are confined entirely to one ganglion. Such neurons are known to be involved in processing mechanoreceptive and proprioceptive information from the thoracic legs of the locust. Discrete populations of spiking local interneurons have been

identified which act as primary integrators of input from cuticular hairs and campaniform sensilla on the legs (Burrows and Siegler 1982; Siegler and Burrows 1983). Individual neurons within these populations have discrete receptive fields on the surface of a leg which may be wholly excitatory, or made up of excitatory and inhibitory regions (Burrows and Siegler 1985). Combinations of these spiking local interneurons synapse with leg motoneurons such that these, too, possess specific receptive fields (Siegler and Burrows 1986). The receptive fields of antagonistic motoneurons are complementary and so tactile stimulation of a particular region of the hindleg can elicit a coordinated local reflex constituting an avoidance response. This coordination is further enhanced because combinations of the spiking local neurons also make inhibitory synapses onto those nonspiking interneurons which would excite motoneurons to oppose the desired reflex (Burrows 1987b). These local reflexes are also called into action during locomotion to enable a leg to step over an obstacle that it may have encountered.

Proprioceptive information from joint receptors in the hindleg is also integrated by these identified populations of spiking local neurons (Burrows 1987a). Furthermore, proprioceptive afferents synapse directly with leg motoneurons so that there are parallel pathways by which this sensory information is processed. These pathways mediate resistance reflexes of the leg (i.e. to counter imposed movements). The function of the interneurons may then be to mediate the inhibitory arm of the reflex by inhibiting antagonistic motoneurons. In addition, they may distribute the integrated information more widely to nonspiking and intersegmental interneurons so as to elicit appropriate coordinated responses in this, and other, legs. There is thus a considerable degree of complexity in the mechanisms of interneuronal integration of sensory inputs and in the circuitry underlying the local reflex adjustments of posture and locomotion in insects.

Intersegmental Interneurons

The vast majority of identified neurons in insects are those classified as intersegmental interneurons, i.e. cells whose axon or axons project from the ganglion in

which they originate. Such interneurons have been described in all of the major sensory pathways and in various motor roles too (see Robertson 1987 for review). A feature that has emerged from studies of the structure and function of intersegmental neurons is that the particular morphology of some classes of these cells can be correlated with the type of connection, i.e. excitatory or inhibitory, they make with other neurons (Pearson and Robertson 1987). Commonly when analyzing systems of neurons in insects, a particular cell's response to sensory stimuli and its discharge pattern during a behavior are known, but information about its connectivity or synaptic action is not. Thus, to be able to predict what a neuron's synaptic function will be based on its structure alone makes it possible to suggest which other neurons it is likely to connect to. This predictive power is of great value when attempting to construct a plausible circuit for a behavior.

Circuitry Underlying Behavior

Although many interneurons in insects have now been associated with particular behaviors (reviewed by Robertson 1987), detailed knowledge of the underlying circuitry exists only for the flight and jumping systems in Orthopterans (crickets, locusts and grasshoppers). More than 50 interneurons are now known to be involved in generating the flight rhythm in locusts and many of the connections between these neurons have been elucidated (Robertson and Pearson 1982, 1983, 1985a,b). Also, much is known of the way in which various proprioceptive inputs from the wings contribute to the generation and shaping of the flight rhythm (e.g. Pearson et al. 1983; Möhl 1985a,b; Reye and Pearson 1987; Wolf and Pearson 1987). In addition, neuronal mechanisms for producing compensatory steering movements during flight have been discovered. Descending sensory signals from the brain which indicate deviations in course influence both pre-motor interneurons and motoneurons in the flight system and are thereby able to bring about corrective steering responses (Reichert and Rowell 1985, 1986; Reichert et al. 1985).

The locust jump, which is the subject of the present investigation, is often cited as the behavior in insects for which there is the most complete understanding of the underlying neuronal mechanisms (reviewed by Pearson 1983; Pearson and O'Shea 1984; Robertson and Pearson 1985b). Jumping is of particular interest because it is a ballistic movement which results from a rather stereotypic motor program. Moreover, there is much evidence to indicate that sensory feedback is necessary for the performance of the program. Before reviewing the neural mechanisms controlling this behavior, some essential background information will be presented.

Locusts jump (and kick) by rapidly extending the tibiae of the hindlegs. If this leg extension were produced only by the rapid contraction of the extensor tibiae muscles (Fig. 1.1), the muscle would not be able to develop its maximum force. Since locusts commonly perform jumps and kicks to escape from threatening situations or to defend themselves, it is necessary that the largest force possible be delivered rapidly. This is achieved by allowing the extensor muscle to develop tension slowly, under isometric conditions, while the leg is held in the fully flexed position by the antagonist flexor tibiae muscle (Brown 1967). This stage of the motor program is called the co-contraction phase (Godden 1975; Heitler and Burrows 1977a). The small flexor muscle is able to resist the force produced by the much larger and more powerful extensor due to a number of structural specializations of the hindleg. Firstly, the geometry of the femoral-tibial joint is such that when the tibia is in the fully flexed position the flexor muscle has a large mechanical advantage over the extensor (Heitler 1974). Secondly, there is a catch mechanism (the lump, Fig. 1.1) associated with the flexor tendon which only becomes operational when the leg is in full flexion. The biomechanical aspects of jumping have been thoroughly investigated (Bennet-Clark 1975). During the prolonged period of flexor and extensor co-contraction the work done by the extensor muscle is stored in the distortion of elastic cuticular elements in the hindleg. Ballistic leg extension finally occurs when this stored energy is rapidly released by the sudden relaxation of the flexor muscle. This has been termed the trigger phase of the program (Heitler and Burrows 1977a). Although this

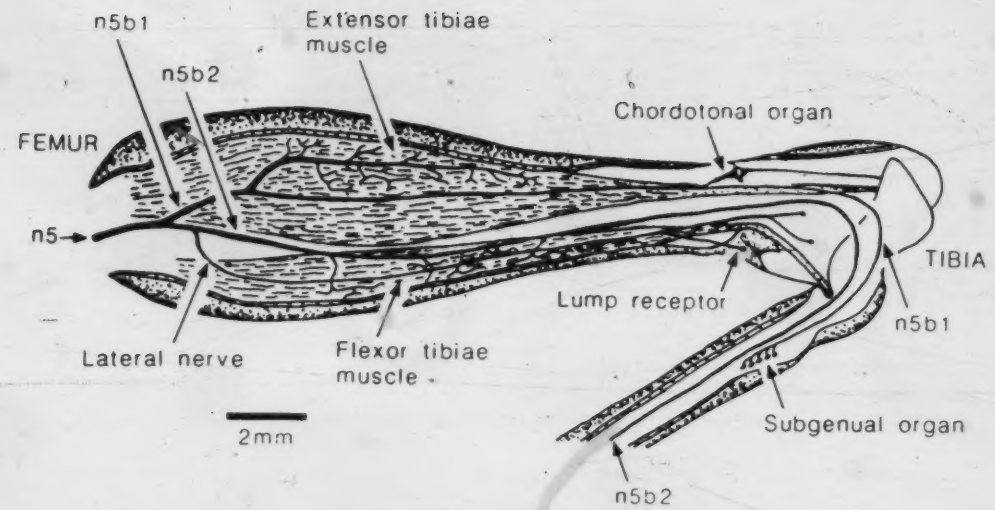

Fig. 1.1. Diagram of the femur and proximal tibia of the hindleg. Only those muscles, nerves and sense organs of particular relevance to the present study are shown (adapted from Heitler and Burrows 1977a).

sequence of events entails a relatively long delay (approximately 500 ms) until the time the movement is produced, this disadvantage is presumably outweighed by the considerable power amplification, and hence velocity amplification, achieved by the mechanical system during the kick or jump (Bennet-Clark 1975; Heitler and Burrows 1977a).

The hindleg extensor tibiae muscle is innervated by two excitatory motoneurons - one fast and one slow motoneuron (Hoyle 1955). The flexor muscle is innervated by nine excitatory and two inhibitory motoneurons (Burrows and Hoyle 1973; Burrows and Horridge 1974). The cell bodies of these motoneurons are all located in the metathoracic ganglion. At the time this investigation was initiated, it was thought that excitation of the fast extensor motoneuron during co-contraction was produced by positive feedback from various proprioceptors in the hindleg (Heitler and Burrows 1977b). Activity in the flexor motoneurons was thought to be maintained by two mechanisms - a central excitatory connection between the fast extensor motoneuron and the flexors (Hoyle and Burrows 1973), and reflex excitation from several hindleg sense organs (Heitler and Burrows 1977b). A very recent study (Heitler and Bräunig 1988) has shown that although these circuits do exist, they are not chiefly responsible for producing the pattern of activity in the motoneurons. This new evidence is first considered in Chapter 5.

The current state of knowledge of the neuronal mechanisms involved in producing kicks and jumps is covered in detail in the introductions of the relevant experimental chapters of this thesis. Here, only a brief review of these mechanisms will be provided. Heitler and Burrows (1977a) concluded that the trigger phase of the kick or jump motor program is produced by the sudden inhibition of the excitatory flexor motoneurons and the excitation of the inhibitory motoneurons. A subsequent study (Pearson et al. 1980) identified a pair of metathoracic interneurons, labeled the M-neurons, whose physiological characteristics and synaptic connectivity indicated they might be responsible for triggering the jump by inhibiting the excitatory flexor motoneurons. Based on these, and later, findings a model was proposed to explain how various exteroceptive and proprioceptive stimuli might summate to produce a

trigger discharge in the M-neurons (Pearson et al. 1980; Steeves and Pearson 1982). The only other interneurons which have been implicated in the control of jumping are the C-neurons (Pearson and Robertson 1981). These mesothoracic interneurons make monosynaptic excitatory connections with hindleg flexor and extensor motoneurons and their function is thought to be to initiate the co-contraction phase in aroused animals.

The present investigation was undertaken to extend the above findings, and, more specifically, to test the validity of the model first proposed by Pearson et al. (1980) to explain how kicks and jumps are triggered. In the second chapter, the involvement of the M- and C-neurons in the motor program is examined by recording from these interneurons during kicks. In addition, many other meso- and metathoracic interneurons are described which may also be involved in producing kicks and jumps. In the third chapter, the importance of the M-neurons was examined by testing whether they are necessary and sufficient for producing trigger activity. The possible role of exteroceptive and proprioceptive inputs in generating the discharge in the M-neuron during the trigger phase was then investigated (Chapters 4 and 5). The mechanisms by which flight motor activity is coupled to the jump were studied in the sixth chapter. Finally, the implications of these new findings are discussed and a revised scheme of the neuronal system underlying jumping and kicking in the locust is presented (Chapter 7).

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II. INTRACELLULAR RECORDINGS FROM INTERNEURONS AND MOTONEURONS DURING BILATERAL KICKS : IMPLICATIONS FOR MECHANISMS CONTROLLING THE JUMP[1]

A. INTRODUCTION

The neuronal mechanisms controlling the locust jump have received considerable attention in past years. The activity of both motoneurons (Godden 1975; Heitler and Burrows 1977a) and interneurons (Pearson, Heitler and Steeves 1980; Pearson and Robertson 1981) during jumping has been examined, as has the involvement of sensory mechanisms in the jump (Heitler and Burrows 1977b; Steeves and Pearson 1982). The jump consists of several distinct phases: 1) an initial flexion of the hindleg tibiae to bring them into the position for jumping, 2) a co-contraction of the antagonist flexor- and extensor tibiae muscles of the hindleg, during which time considerable force is generated by isometric contraction of the large extensor muscle, and 3) a sudden inhibition of the flexor activity allowing the tibiae to rapidly extend due to release of the energy stored in elastic elements of the femoral-tibial joint. These same phases are evident during kicking (Heitler and Burrows 1977a).

Currently, the proposed neuronal circuitry controlling kicking and jumping (see Pearson 1983) involves only two pairs of thoracic interneurons, the C- and M-neurons. The C-neurons are considered to cause the initial locking of the tibiae into full flexion by synchronously activating the flexor and extensor muscles at the onset of co-contraction (Pearson and Robertson 1981). As yet, however, no recordings have been made from C-neurons during the rest of co-contraction leading to either a kick or a jump. The M-neurons are thought to be responsible for the sudden inhibition of flexor activity which triggers the jump (Pearson et al. 1980). The M-neurons make monosynaptic inhibitory connections with flexor motoneurons and receive depolarizing input resulting from visual,

[1]A version of this chapter has been published. Gynther IC, Pearson KG (1986) J Exp Biol 122:323-343

auditory and tactile stimuli, all of which are quite effective in eliciting jumps from intact animals. However, because the M-neurons have a characteristically high threshold for action potential generation, they are rarely induced to spike even upon simultaneous presentation of such stimuli. The M-neurons also receive depolarizing input from hindleg proprioceptors and it has been proposed that the gradual increase in the strength of this input during co-contraction brings each M-neuron closer to its threshold for spiking, thus allowing it to discharge action potentials in response to other sensory stimuli (Steeves and Pearson 1982). According to this idea, the proprioceptive information gates M's activity and ensures that each M-neuron will discharge (consequently inhibiting the flexors and triggering the jump) only when sufficient force has been generated within the appropriate femur.

The main purpose of the present study was to examine more closely the proposed roles of the C- and M-neurons by recording from the neuropile processes of these cells during the production of bilateral hindleg kicks. In addition, the pattern of synaptic input to hindleg motoneurons was examined and recordings from other interneurons within the thoracic ganglia were made to determine whether some of these may also play a role in producing the kick. Further evidence is presented which supports the idea that the M-neurons are the trigger neurons for the jump. However, their activity is not gated in a simple manner by proprioceptive feedback as proposed in earlier studies. This, together with the finding that other interneurons are strongly activated during a kick, indicates that the circuitry controlling the jump is not as simple as has previously been proposed.

B. MATERIALS AND METHODS

All experiments were performed on adult male and female *Locusta migratoria* reared in a long-established colony at the University of Alberta. The sex of an animal had no influence on the results of this study. Experiments were carried out at room temperature (22-24°C).

EMG Recording from Intact Animals

Details of the procedure used to record flexor- and extensor tibiae muscle activity in intact locusts have been described previously (Pearson and Robertson 1981). Briefly a pair of 50 µm copper wires, insulated except for their tips, was implanted into the extensor muscle of one hindleg. Flexor activity could still be recorded in this manner and was evident as the smaller amplitude activity in our recordings. The animal was then tethered to an overhead support by way of a cotton thread attached to the pronotum. This arrangement permitted the locust freedom of movement over an area of about 50 cm diameter. Jumps either occurred spontaneously or were elicited by making loud noises or by lightly touching the animal's body.

Preparation of Animals

Animals were pinned dorsal side up on a cork board and their hindleg femora fixed firmly in place with plasticene. The tibiae of these legs, cut so that only their proximal third remained intact, were usually allowed unrestricted movement. However, for some experiments a metal rod, mounted on a manipulator, was used to prevent full flexion of one of the tibiae. A combined myogram of the flexor and extensor tibiae muscle activity of each hindleg was obtained by implanting a pair of 200 µm copper wire electrodes into the extensor muscles. These electrodes could also be used to antidromically stimulate the fast extensor tibiae motoneuron. The thoracic ganglia were exposed by removing the gut, ventral diaphragm and overlying muscles (see Pearson et al. 1980 for details of the dissection). In the majority of experiments, nerves 3 and 4 of both the meso- and metathoracic ganglia were cut so as to denervate most of the thoracic flight musculature and thereby improve the stability of the preparation. These ganglia were then supported on a rigid stainless steel plate and kept covered with locust saline (in mM: NaCl,147; KCL,10; $CaCl_2$,4; NaOH,3; HEPES,10).

Bilateral kicks of the hindlegs were evoked by gently stroking or pinching the animal's abdomen, mouthparts or antennae with a pair of forceps. It was possible to evoke kicks from approximately 80% of these fully dissected animals. A kick could easily be distinguished from

rapid tibial extension because continued simultaneous activity of flexor and extensor muscles (co-contraction) did not occur during the latter, and because kicks produced an audible "click". Instability of intracellular recordings during kicking was generally not a problem. It was only rarely that the violent movement of the tibiae during kicking dislodged a microelectrode from within a cell. All electrical recordings were stored on magnetic tape and later displayed on a Gould ES1000 chart recorder.

Intracellular Recording and Dye-filling

All recordings from motoneurons and interneurons were made by penetrating their major neuropile processes. Glass microelectrodes were filled with either 1M potassium acetate or a 5% aqueous solution of the dye Lucifer Yellow. Lucifer staining enabled the morphology of a penetrated neuron to be determined following recording of its physiology. After staining, the ganglia were fixed for 30 minutes in a 4% solution of paraformaldehyde, dehydrated in alcohol and then cleared in methyl salicylate for 15 minutes before being viewed as wholemounts.

The criteria for recognizing penetrations of fast extensor motoneurons (FETi), flexor motoneurons, and M- and C-neurons have been described previously (Pearson et al. 1980; Pearson and Robertson 1981). The cells could be recognized so reliably that dye-filling was not necessary for their identification. All other inter- and motoneurons, however, were always stained following recording.

C. RESULTS

Motor Patterns During Jumps and Bilateral Kicks

Although jumping and defensive kicking in locusts are related behaviors, there are distinct differences between them. One of the more obvious is that jumping involves both metathoracic legs whereas kicking usually involves just one. Another is that, before jumping,

a freely moving locust assumes a crouched position with both hind coxae depressed, such that the femora lie parallel to the substrate. When the tibiae extend during the jump, force is directed downwards and backwards (Godden 1969, 1975). Defensive kicks, however, can be accurately directed towards a source of irritation by rotation of the leg about the thoraco-coxal joint. Here the force of the extending tibia is often directed upwards and backwards. There has been no comprehensive EMG study of all muscles involved in jumping and kicking, and consequently a detailed account of the differences between the motor programs for these two behaviors is not available. However, in as far as the activity of the metathoracic flexor- and extensor tibiae muscles is concerned, there is good evidence to suggest that the motor programs for jumping and kicking in the locust are very similar (Godden 1975; Heitler and Burrows 1977a; Pflüger and Burrows 1978). From here on in this thesis the term "motor program for a jump or kick" is used to mean only this pattern of flexor and extensor activity.

The preparation for intracellular recording in the locust involved such an extensive dissection that it was necessary to question whether the behavior elicited from these dissected animals resembled anything seen in intact, freely moving locusts. A comparison of the myograms recorded during jumps by intact animals and restrained kicks by dissected animals showed that the main features of the motor patterns for these behaviors were very similar (Fig. 2.1A) and closely resembled those described in detail by Heitler and Burrows (1977a). Typically, a period of flexor activity of about 50-500 ms duration was followed by co-contraction of the flexor and extensor muscles, as indicated by a period in which spikes occurred in both muscles. Spikes in the extensor muscle EMG were due to the activity of the single fast extensor motoneuron innervating that leg. Co-contraction lasted for a period of some 300-800 ms (mean for a jump=340 ms, mean for a kick=450 ms) and ended with a sudden cessation of flexor activity. Usually extensor activity continued for a short period (about 20 ms) after the termination of activity in the flexors.

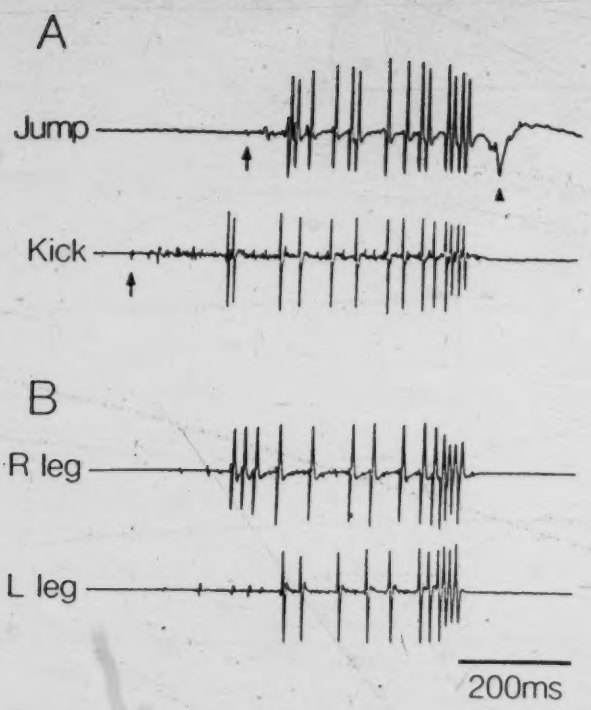

Fig. 2.1. Electromyographic recordings from hindleg flexor and extensor tibiae muscles of locusts during jumps and bilateral kicks. A Top trace - EMG from an intact locust during an unrestrained jump; bottom trace - EMG recorded from one leg during a bilateral kick by a locust dissected for intracellular recording. The large spikes in both traces were due to activity in the single fast extensor tibiae motoneuron. The small amplitude spikes were caused by activity in the flexor tibiae muscle. Except for the shorter duration of the sequence during jumping, the motor programs for kicking in the dissected animal and jumping in the intact animal were similar. Each began with a period of flexion (commencing at arrows) followed by a co-contraction of both the flexor and extensor muscles. As illustrated here, the motor program often ended with a rapid burst of 4-7 spikes in the extensor muscle. The movement artifact in the top trace (arrowhead) was caused by the animal jumping. B EMG recordings from a dissected locust during a bilateral kick of the hindlegs (top trace - right leg; bottom trace - left leg). All the features of the motor program are as described in A. Note the simultaneous burst in the extensor muscle of each leg which terminated the kick sequence. Note also that the kicks were triggered simultaneously in the two legs as indicated by the synchronous cessation of muscle activity.

Although the femora of the hindlegs were held fixed so that they could be neither depressed nor rotated upwards, as would normally occur during a jump or kick, the great majority of kicks elicited from the dissected animals involved the synchronous extension of both hind tibiae (Fig. 2.1B) rather than just one as is usual for a defensive kick. This, together with the basic similarity between the patterns of motor activity seen in intact and dissected animals, indicated that the behavior observed in dissected locusts resembled jumping more than it did defensive kicking. For this reason it may be more correct to refer to this behavior as fictive jumping. However, in order to retain consistency with the terminology of earlier studies (e.g. Heitler and Burrows 1977a,b) this fictive jumping is referred to as kicking throughout the thesis. It should be borne in mind then that, if there is any difference between the neural mechanisms for jumping and kicking, the results of this study are likely to have more direct relevance to the jump system than to the system underlying defensive kicking.

Despite their overall similarity, there were minor differences between the motor programs seen during kicking in dissected animals and jumping in intact animals. The total duration of the program for an intact animal's jump was usually shorter than that for kicking in a dissected animal. This was due to shorter periods of both initial flexion and co-contraction in the intact animal. In some highly aroused locusts, co-contraction began not with an initial activation of flexor motoneurons alone but with synchronous activation of FETi and flexor motoneurons (see Pearson and Robertson 1981). This was never observed in dissected animals.

A major difference between the motor programs recorded in this investigation and those reported by others was that the extensor muscle commonly displayed two phases of activity. Throughout most of co-contraction spikes occurred in this muscle at a more or less constant frequency of 10-40 spikes/s and on a few occasions almost 60 spikes/s (Fig. 2.2D). Sometimes the muscle spikes occurred in discrete groups of 2-3, but with their overall frequency still in the 10-40 spikes/s range. The final phase of co-contraction, however, usually involved a higher frequency of activity in the extensor muscle just prior to the jump

or kick (Fig. 2.1A,B). The timing of this final burst was variable. Usually it commenced before, and extended just beyond, the termination of flexor activity. Occasionally it began after flexor activity had ceased. The number of spikes during the final extensor burst was typically 4-7 and they occurred with a frequency of 70-130 spikes/s. A noticeable feature was that the burst often began synchronously in both legs (Fig. 2.1B).

Recordings from Motoneurons During Bilateral Kicks

The motoneurons driving the power-producing extensor and power-controlling flexor muscles of the hindleg constitute the output stage of the neuronal circuitry controlling the jump. As such, a careful analysis of the synaptic input that these motoneurons receive during bilateral kicking can reveal much about the underlying mechanism of the jump. Presented here are recordings, made during kicking, from the neuropile processes of the fast extensor tibiae motoneuron and a variety of the nine excitatory flexor motoneurons. All previous recordings (Heitler and Burrows 1977a,b) have been made from neuronal somata and consequently provide an attenuated view of synaptic events.

Fast Extensor Tibiae Motoneurons

The pattern of activity seen in FETi during kicking was variable. During weak kicks, FETi displayed a slight plateau of depolarization interrupted by partial membrane repolarizations (Fig. 2.2A). No final, higher frequency burst occurred during these kicks. More commonly, FETi displayed the same biphasic pattern that was often observed in the EMG recordings of extensor muscle activity, with a period of relatively low frequency spiking followed by a rapid burst of action potentials in the cell. During the first phase of co-contraction, action potentials could occur at either a fairly constant frequency or in groups of 2-3, with the groups separated by roughly constant intervals. Two such biphasic patterns were evident. In one, FETi showed a plateau depolarization throughout most of co-contraction before receiving a marked, often pulse-like, depolarization accompanied by a rapid burst of 4-7 spikes just preceding the kick (Fig. 2.2B). In the

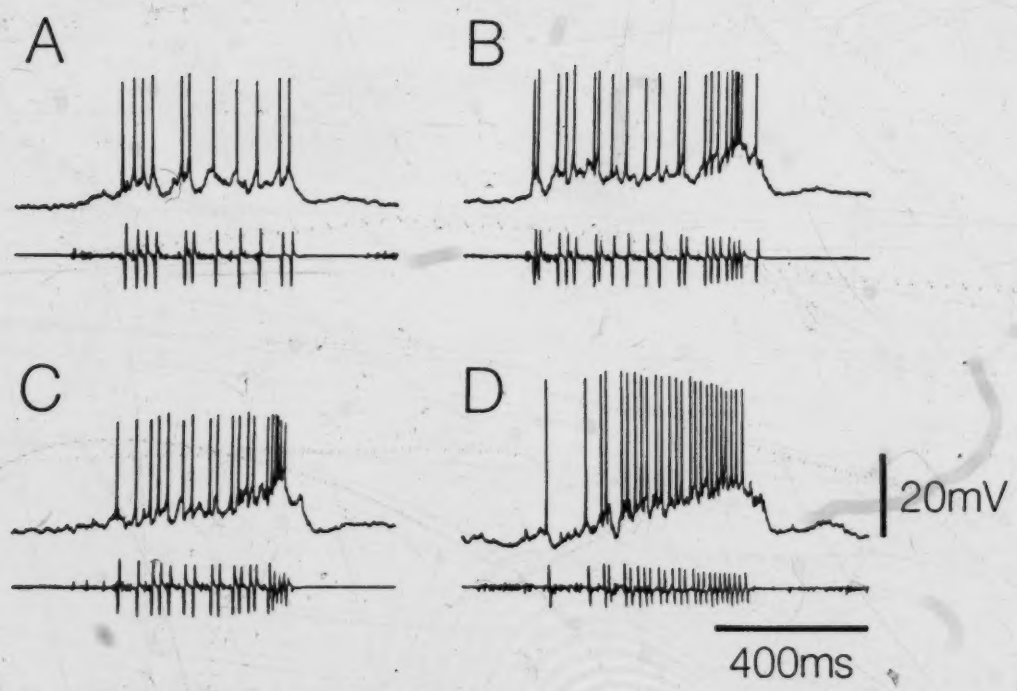

Fig. 2.2. Intracellular recordings from the fast extensor tibiae motoneuron during kicks. Top traces - intracellular recordings; bottom traces - EMGs from flexor and extensor tibiae muscles of the innervated legs. A A weak kick in which no final burst occurred in the motoneuron. B,C Examples in which FETi's activity concluded with a marked depolarization and rapid burst of spikes. D A sequence in which FETi received a ramp depolarization throughout co-contraction and fired at higher than normal frequencies. The patterns of activity shown in B,C were those most commonly observed.

other, it received a weak ramp-like depolarization throughout co-contraction leading to the distinct final burst (Fig. 2.2C). In both cases, the duration of the higher frequency burst of spikes was in the range of 30-100 ms. Another pattern of FETi activity seen in one animal during co-contraction consisted of a much stronger ramp depolarization which did not end with a rapid burst of action potentials (Fig. 2.2D). In these cases, the spike frequency was usually higher than normal throughout co-contraction. At times the ramp could be interrupted by marked membrane repolarizations. Except for this last example, each of the described patterns of activity could be observed in a single preparation, but the patterns shown in Fig. 2.2B,C were the most common. The boundaries between the various patterns were not so clear cut as to suggest functionally separate motor programs.

The recordings do suggest, however, that FETi could receive two distinct inputs: 1) a tonic or ramp-like excitation throughout most of co-contraction and 2) a final phasic excitation just preceding the kick. This conclusion was supported by observations of the pattern of synaptic input to FETi when kicks were elicited from the contralateral leg but prevented in the ipsilateral leg. (It was possible to prevent one hindleg from kicking by placing an obstruction between the tibia and femur so that the leg was unable to flex fully.) Throughout most of co-contraction of the opposite leg, FETi innervating the obstructed leg was often quiescent. On no occasion was it continuously depolarized and active during co-contraction of the opposite leg as it normally would have been. This suggested that FETi's normal pattern of activity during the first part of co-contraction was not due to a bilaterally symmetrical central excitation but more probably to a sensory source, as proposed by Heitler and Burrows (1977b). However, the ipsilateral FETi was consistently depolarized above threshold at, or near, the time of the final burst of spikes in the opposite leg. This depolarization was usually weak with only one or two spikes resulting (see Fig. 2.5), but sometimes as many as six occurred. As mentioned, the exact timing of this input was variable. In most cases it coincided with the burst in the opposite FETi, but in others it occurred just before this burst started or just after it had finished.

Flexor Tibiae Motoneurons

Each flexor tibiae muscle of the locust is innervated by nine excitatory motoneurons (Burrows and Hoyle 1973), which can be classified physiologically as fast, intermediate or slow flexor motoneurons. Although no attempt was made to record from all nine flexors in this study, numerous recordings during kicking were made from neurons in each group. There is a basic similarity between the groups in their patterns of activity and because of this it was felt that a complete catalogue of flexor motoneuron responses was not necessary.

Two examples of flexor activity during kicking are shown in Fig. 2.3. A striking feature, seen in all flexor motoneurons, was the large plateau depolarization they received throughout co-contraction. The initial depolarization leading to this plateau could occur gradually, in a ramp-like manner (Fig. 2.3A), or more quickly, following several summating excitatory postsynaptic potentials (EPSPs)(Fig. 2.3B). In some motoneurons, the rise time of this plateau was so rapid that it became difficult to distinguish the individual EPSPs. It is important to note that the flexors received this initial depolarization independently of activity in FETi, as indicated by the absence of extensor spikes in the myogram at the time the flexors became active. Consequently the central excitatory connection that exists between FETi and the flexor motoneurons (Hoyle and Burrows 1973) could not have been responsible for initiating flexor activity. This connection probably does contribute some excitation to the flexors during co-contraction, however, and may account for the oscillations in membrane potential sometimes seen superimposed on the plateau depolarization in recordings of flexor activity. These oscillations corresponded on a 1:1 basis with FETi spikes seen in the accompanying myogram. This correspondence declined, however, as the co-contraction sequence continued, most probably because of a rapid decrease in the strength of the central FETi-flexor interaction which occurs with repeated spike activity in the fast extensor tibiae motoneuron (Heitler and Burrows 1977b).

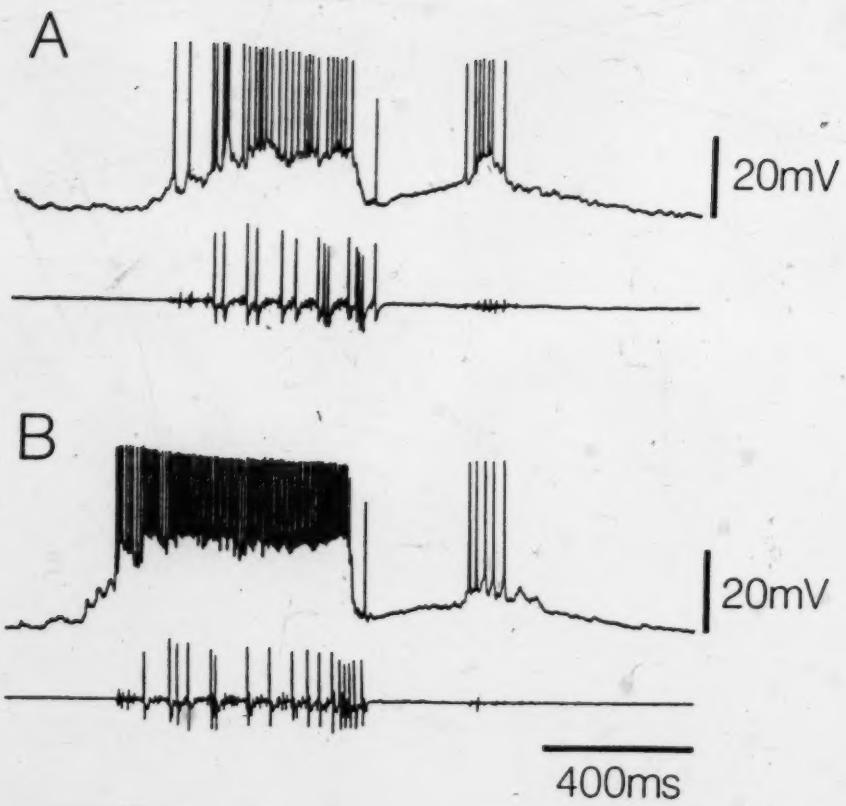

Fig. 2.3. Intracellular recordings from flexor tibiae motoneurons during kicks. Top traces - intracellular recordings; bottom traces - EMGs from flexor and extensor tibiae muscles of the innervated legs. **A** Intermediate flexor motoneuron. **B** Slow flexor motoneuron. Note the inhibition of the flexors (particularly marked in **B**) which just preceded the termination of extensor activity and triggered the kick. The second burst of spikes in each flexor motoneuron, 200-300 ms after the kick, caused the tibia to re-flex.

The frequency at which flexors spiked during the plateau depolarization was related to their physiological category, as has been reported by Heitler and Burrows (1977a). Fast and intermediate flexor motoneurons commonly discharged at 70-90 spikes/s during co-contraction (Fig. 2.3A) whereas slow flexors reached discharge frequencies of 200 spikes/s or more (Fig. 2.3B).

The most important feature observed in all flexors during kicking was their rapid inhibition which terminated co-contraction and triggered the kick. Its timing relative to the end of extensor activity in the ipsilateral leg was variable, occurring over a range of between 4 and 67 ms prior to the last spike in the EMG. So, too, the timing of flexor inhibition relative to the start of the final high frequency burst in the ipsilateral FETi was variable. Examples have been recorded in which it occurred as much as 20 ms before the start of the final burst in FETi. However, it was more usual for the inhibition to occur after this burst had commenced and while it was still in progress (mean=15.7 ms following the first FETi spike of the burst). This degree of variability in timing of the two phenomena - the flexor inhibition and the final FETi excitation - suggested that these events did not have a common underlying origin.

Recordings from Interneurons During Bilateral Kicks

To gain more insight into the cellular mechanisms for patterning motor activity for the jump, the activity of numerous identified interneurons in the meso- and metathoracic ganglia was recorded during bilateral kicks. Initial efforts concentrated on the C- and M-neurons because they are known to be intimately involved in the mechanisms by which the jump motor program is initiated and by which the jump is triggered, respectively (Pearson and Robertson 1981; Pearson et al. 1980). No attempt was made to elucidate the connections between these and other interneurons.

The M-neurons

Intracellular recordings from the M-neurons revealed two previously unreported features : 1) throughout the period of initial flexion and the subsequent co-contraction the M-neurons were hyperpolarized by several millivolts, and 2) at the end of co-contraction they received a strong, pulse-like depolarization causing them to discharge at extremely high frequencies.

It has been reported that the M-neurons (Fig. 2.4A) are gradually depolarized during co-contraction and then, due to the occurrence of visual and auditory stimuli, are caused to discharge in a burst (Steeves and Pearson 1982). In contrast, no slow depolarization was ever observed in an M-neuron prior to a kick. Instead, a hyperpolarization of up to 6 mV was consistently noted (Fig. 2.4B,C). The magnitude of this hyperpolarization depended on both the site and quality of the intracellular penetration. In recordings from the central processes of the cell (Fig. 2.4B,C) the hyperpolarization was very obvious. In recordings from the transverse process, proximal to the point where it bifurcates into the ascending axon and the large lateral branch, the hyperpolarization was less marked (Fig. 2.4D), as was also true in cases where the cell had been injured on penetration. It was possible that this pattern of activity in the M-neuron may not have been the same as that during kicks in the intact animal, due to the absence of proprioceptive information from afferent fibres projecting from the hindleg femur and tibia to the central nervous system (CNS) via nerve 3 (in most experiments this nerve was cut). However, these same features of M's activity were evident even when all peripheral nerves were left intact. For this reason the recordings presented here are considered to be typical of the M-neuron's normal activity.

The initial flexion movements of the two hind tibiae did not always occur synchronously. Flexion in one leg could sometimes precede the other leg by as much as 500 ms. The onset of the hyperpolarization in an M-neuron appeared to coincide with the beginning of flexor activity in whichever leg was first to flex. Thus, it was not always

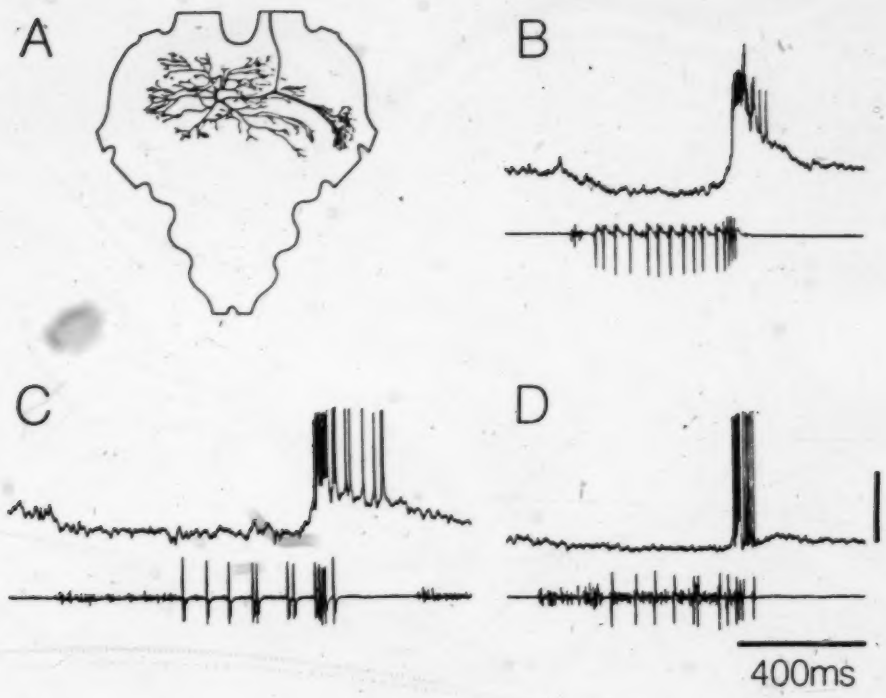

Fig. 2.4. Intracellular recordings from the M-neuron during kicks. Top traces - intracellular recordings; bottom traces - EMGs from hindleg flexor and extensor muscles on the side to which the M-neuron sent its major branch. A Diagram of M's structure within the metathoracic ganglion. B,C Recordings from central processes during kicking. The exact sites of penetration were not determined but judging from spike amplitudes, the recording in B was from a finer central process than was the recording shown in C. D Recording from the transverse process of M, proximal to the point where it bifurcates into the ascending axon and the large lateral branch. The recording site was quite close to the spike-initiating zone. The M-neuron fired at frequencies of 250-400 spikes/s during its burst (the individual spikes cannot be discerned here). Note the hyperpolarization (evident in B,C, but less so in D) which commenced at or near the time of tibial flexion. Scale bar, 10 mV in B and D, 20 mV in C.

coincident with flexion on the side to which the M-neuron sent its major branch (which here is termed the ipsilateral side though it is actually the side contralateral to M's soma). The duration of the hyperpolarization in M was variable, being dependent on the length of the initial flexion and co-contraction periods combined.

The depolarization that each M-neuron received just prior to a kick could occur even in the complete absence of visual, auditory and tactile stimuli. It was triphasic in form, with a rapid ramp depolarization leading to a pronounced pulse-like excitation, followed by a slow ramp repolarization (Fig. 2.4B,C). The pulse phase usually lasted 40-90 ms, during which time the cell discharged at frequencies of 250-400 spikes/s. It was common for an M-neuron to continue spiking as its membrane gradually repolarized.

As was suggested by the variability in timing of the flexor motoneuron inhibition relative to the end of extensor activity, the timing of the M-neuron's burst relative to the last FETi spike was also variable. In an analysis of 42 kicks by five animals the first spike in M preceded the last spike in the ipsilateral extensor EMG by an average of 23.4 ms (SD=15.7 ms). The greatest separation observed was 60.4 ms. In two kicks M's discharge followed the last extensor spike by periods of 4.0 and 6.4 ms, respectively. These were the only two occasions on which M became active after extensor activity had ceased. In these cases, flexor EMG activity was also observed following the last FETi spike. During kicks in which FETi's activity ended with a rapid burst, the ipsilateral M was observed to begin firing before, during, or even after this burst. This variability in the timing of the M-neuron's activity was present even during repeated kicks by the same animal. Again it indicated that the M-neuron was not responsible for the final burst in FETi. However, the correlative evidence of the range of times of flexor shutdown relative to the end of extensor activity as compared with the timing of the burst in each M-neuron was entirely consistent with the idea that the M-neurons provided the trigger inhibition to the flexor motoneurons at the end of co-contraction.

In an attempt to determine whether the pattern of input each M-neuron received during kicking was of a central, bilateral origin or whether it depended on incoming sensory information from the ipsilateral leg, recordings were made from an M-neuron whilst preventing the ipsilateral leg from kicking. A kick was then elicited from the opposite leg. In this situation the M-neuron displayed the same pattern of activity - initial hyperpolarization followed by a triphasic depolarization - that it would have if the locust had been able to kick its ipsilateral leg (Fig. 2.5). This result indicated that the input to the M-neurons during kicking was bilateral and that each M-neuron was able to produce its trigger discharge without the usual proprioceptive information from the ipsilateral leg. However, although the overall pattern of activity was normal, it appeared that the spike frequency attained during M's discharge was not as high as when both legs were able to kick (compare Figs. 2.5 and 2.4C). Insufficient data were obtained to quantify this, but it may be that the ipsilateral proprioceptive input directly or indirectly affects the intensity of the M-neuron's burst.

The C-neurons

In intact animals the C-neurons are thought to initiate the motor program for a jump by synchronously activating FETi and flexor motoneurons at the very start of co-contraction. This brings the hindlegs into the correct position for jumping (Pearson and Robertson 1981). Co-contraction is a separate phase of the motor program which then follows. Because of the lower levels of arousal in dissected animals, this synchronous activation of FETi and flexors at the start of co-contraction never occurs. Instead, flexor activity may precede the start of extensor activity by as much as 500 ms. This proposed role of the C-neurons therefore cannot be tested using intracellular recording techniques. Rather, their activity during the remainder of the kick motor program (i.e. the rest of co-contraction and the trigger activity) was studied to determine whether they might contribute to other aspects of the jump.

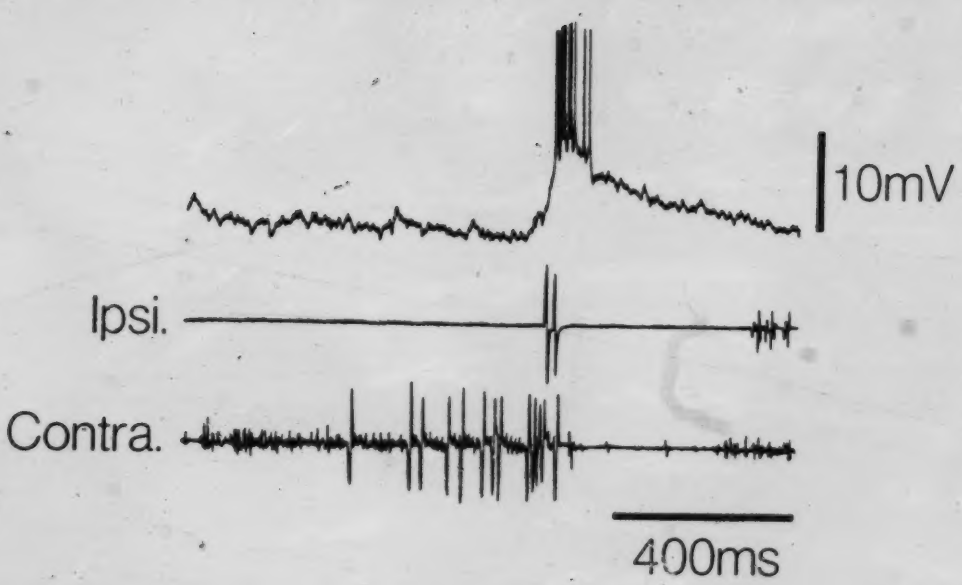

Fig. 2.5. Intracellular recording from an M-neuron made while the ipsilateral leg (i.e. on the side to which the M-neuron sent its major branch) was prevented from fully flexing and therefore could not kick. A kick was elicited from the contralateral leg. Top trace - intracellular recording; middle trace - EMG from the ipsilateral leg; bottom trace - EMG from the contralateral leg. Note that M's pattern of activity was unaltered by obstructing its ipsilateral leg. Note also the two spikes in the extensor muscle of the ipsilateral leg (caused by activity in FETi) which occurred close to the time of the extensor burst in the unobstructed leg.

Commencing sometime during the initial flexion of the leg or co-contraction itself, the C-neurons (Fig. 2.6A) received a slow ramp depolarization. The spike frequency usually increased during this depolarization, reaching 100-200 spikes/s just before the cell was rapidly inhibited (Fig. 2.6B). From the EMG records alone it was not possible to determine whether this inhibition was coincident with that which occurred in the flexor motoneurons.

Because the burst in C corresponded to the burst in FETi and because C makes a strong excitatory connection to FETi (Pearson and Robertson 1981), the C-neurons may have been responsible for generating the final, rapid burst in FETi just prior to the kick. However, several observations did not support this. During some kicks C did not have such a well-defined burst of spikes but instead discharged at a more or less constant frequency throughout the ramp, even though the accompanying EMG record still showed the rapid FETi burst concluding the co-contraction. During one kick (not shown), in which the C-neuron only fired spikes when at its maximum depolarization, this discharge preceded the rapid FETi burst by 90 ms. Moreover, C was then inhibited more than 10 ms before the last spike occurred in the FETi motoneuron and so could not be solely responsible for the continuing extensor burst. The most convincing observation, however, was that locusts were still able to kick when the meso/metathoracic connectives had been cut (see also Godden 1975). The motor program for kicking was not altered significantly by the severing of these connectives, or the abdominal connectives, posterior to the metathoracic ganglionic mass. Thus, even without the descending input from the C-neurons, the rapid burst often occurred in FETi. This observation that kicking can still occur after cutting the meso/metathoracic connectives also clearly demonstrates that the C-neurons are not necessary for the generation of co-contraction as a whole. This was indicated too by the finding that C usually begins to spike only late in the co-contraction phase (Fig. 2.6B).

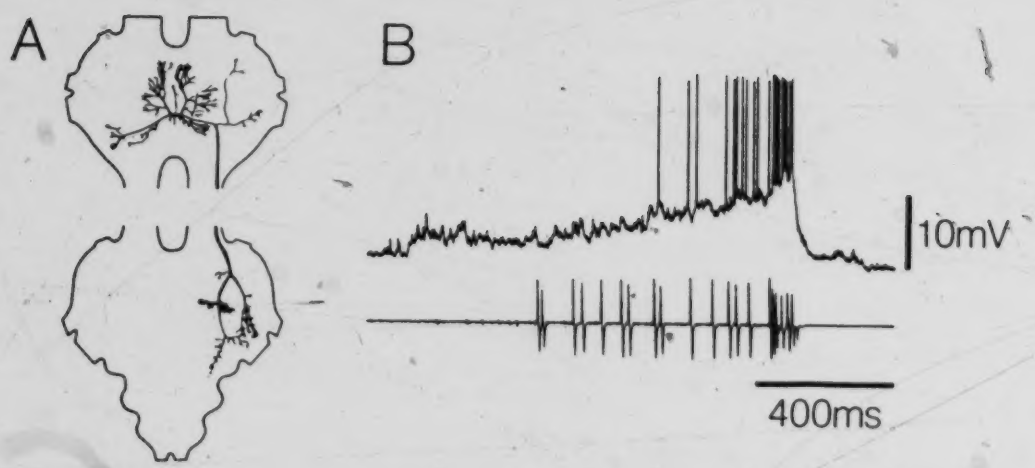

Fig. 2.6. Intracellular recording from the C-neuron during a kick. A Diagram of C's morphology in the meso- and metathoracic ganglia. B Recording during a kick. Top trace - intracellular recording; bottom trace - EMG from the flexor and extensor tibiae muscles on the side to which the C-neuron projected. The recording was made from the large transverse process in the midline of the mesothoracic ganglion.

It would not be inconsistent to suggest that the M-neurons were responsible for the rapid inhibition of the C-neurons, based purely on the timing of this event. Clearly, investigation of this proposal must await simultaneous recordings from the C- and M-neurons. From anatomical evidence alone, however, it appears most unlikely that M would inhibit C because the M-neurons do not possess output branches in those regions of the mesothoracic ganglion where C is thought to receive its input.

Other Interneurons

Recordings from the M-neurons suggested that some interneuronal trigger system may account for the pattern of activity seen in M during kicking. With this in mind, the meso- and metathoracic ganglia were searched for cells which might be members of this trigger system. To date this has revealed 11 interneurons which discharged either during co-contraction or at the time the kick was triggered. Here the characteristics of some of these interneurons are described. Another interest was to find cells which may provide excitatory input to the C-neurons.

The responses of two mesothoracic interneurons during kicking are illustrated in Fig. 2.7. The cell in Fig. 2.7A displayed a characteristically high level of synaptic activity and responded to input from several sensory modalities. It received small EPSPs from the descending contralateral movement detector (DCMD) and was strongly depolarized by high frequency sounds and by tactile stimulation of the abdomen. During co-contraction it was depolarized in a ramp fashion and reached high discharge frequencies (around 200 spikes/s) before being inhibited prior to the kick (Fig. 2.7B). The M-neurons were not responsible for the inhibition of this neuron since flexor activity continued beyond the last spike in the cell. Also, the intensity of its inhibition was not of the same degree as that seen in flexors. This is the only interneuron that has been consistently found to discharge strongly throughout co-contraction.

Another interneuron that received input from DCMD and auditory and tactile stimuli is shown in Fig. 2.7C. The similarity of its inputs, discharge pattern and some

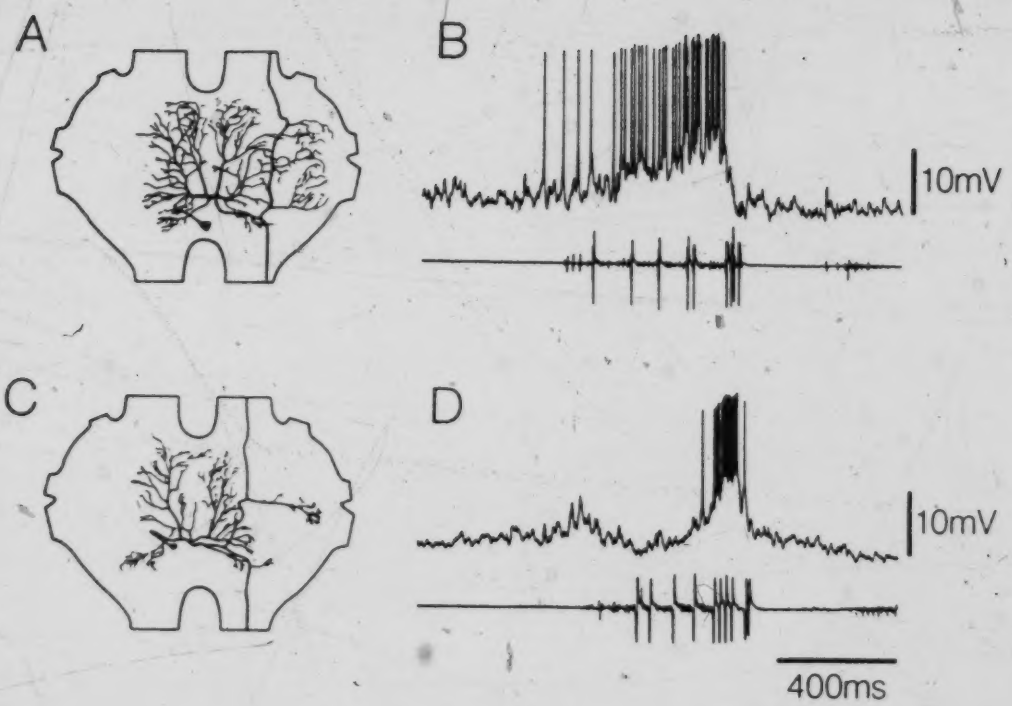

Fig. 2.7. Intracellular recordings from two mesothoracic interneurons during kicks. Top traces - intracellular recordings; bottom traces - EMGs from the hindleg flexor and extensor tibiae muscles on the side to which the interneurons projected. A,B Diagram of one interneurones structure and its pattern of activity during kicking. C,D Diagram of another interneuron and its discharge pattern during a kick. This cell fired at frequencies of about 200 spikes/s during its burst.

features of its anatomy (such as soma position within the ganglion, looped neurite leading from the soma, general position of its central branches, variability in structure of the large central processes and possession of a distinct lateral branch) with those of M suggest that it may possibly be the mesothoracic homologue of the M-neuron. (The presence of a descending axon in a mesothoracic cell and the absence of one in a metathoracic cell does not indicate a lack of homology between the two neurons - see Pearson, Boyan, Bastiani and Goodman 1985.) This cell received a very strong depolarizing input during the final phase of co-contraction and at about the time M would be expected to discharge (Fig. 2.7D). High spike frequencies (also near 200 spikes/s) occurred during this depolarization.

At present seven interneurons within the metathoracic ganglion have been identified which also discharged in bursts prior to a kick (see Fig. 2.8A,C). These cells displayed similar patterns of activity during kicks, with their peak depolarizations occurring 10-30 ms before the last extensor spike in the accompanying EMG (Fig. 2.8B,D). The cell in Fig. 2.8A also received strong DCMD and auditory input.

In addition, certain spiking local interneurons in the metathoracic ganglion were found to discharge strongly at the end of co-contraction. The cell illustrated here (Fig. 2.9A) received a ramp-like depolarization and fired a high frequency burst of spikes (about 200 spikes/s) just preceding, and during, the final burst in FETi (Fig. 2.9B). It was then rapidly inhibited. It was also noted that the injury discharge that accompanied the initial penetration of this neuron caused rapid and maintained extension of the ipsilateral hind tibia. Consequently this local interneuron may play a role in generating the rapid burst in FETi.

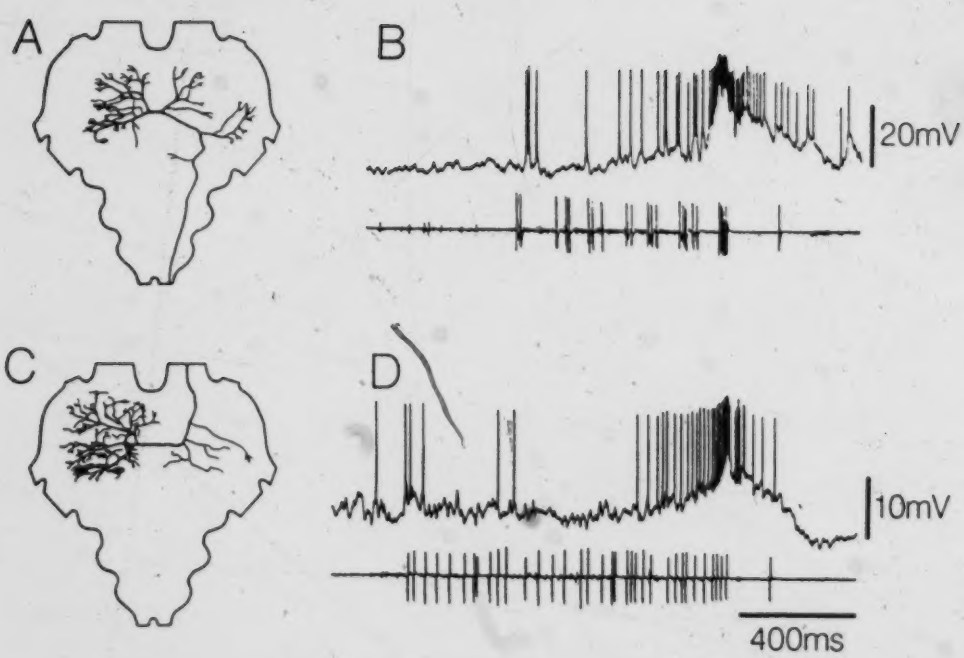

Fig. 2.8. Intracellular recordings from two metathoracic interneurons during kicks. Top traces - intracellular recordings; bottom traces - EMG recordings from the flexor and extensor tibiae muscles on the side which the cells had their predominant output. **A,B** Diagram of the anatomy of one interneuron and its activity pattern during kicking. **C,D** Structure of another interneuron and its response during a kick.

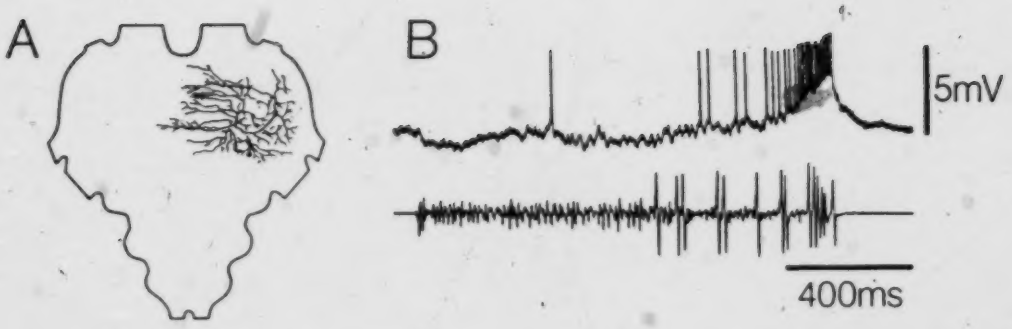

Fig. 2.9. Intracellular recording during kicking from a local interneuron in the metathoracic ganglion. A Diagram of the structure of the interneuron. B Recording of the cell's discharge pattern during a kick. Top trace - intracellular recording; bottom trace - EMG from the flexor and extensor tibiae muscles of the leg ipsilateral to the cell.

D. DISCUSSION

The major finding of this investigation is that the pattern of synaptic input to the M-neurons (the jump trigger neurons) is not consistent with earlier models of the jump circuitry, and instead suggests that there is a higher order trigger system which is activated just prior to a kick or jump. Support for this proposal comes from the identification of numerous interneurons which discharge either during co-contraction or at the time a kick is triggered. In this section this new evidence is examined and its implications for mechanisms triggering the jump are discussed. First, however, the motor program for jumping and kicking is reviewed and some additional features of the program not described in previous studies are considered.

The Motor Program

The major features of the motor programs for kicking in the dissected locust and jumping in intact locusts are similar. In each the program commences with a period of flexor activity, 50-500 ms in duration, followed by a longer (300-800 ms) period of co-contraction of the flexor and extensor muscles (Fig. 2.1A). Flexor activity then ceases due to a rapid inhibition and the kick or jump follows. Godden (1975) has described these same features in jumping locusts, and Heitler and Burrows (1977a) for kicks in animals less radically dissected than our preparation.

The program for jumping does differ to a minor degree from kicking in that the periods of flexion and co-contraction, and consequently the duration of the entire program, tend to be shorter. This was also noted by Pflüger and Burrows (1978). Another difference is that in intact animals the motor program is often initiated by synchronous activation of the flexor and extensor motoneurons (Pearson and Robertson 1981). In contrast, this never occurs in dissected locusts, presumably because of the animals' low levels of arousal. These differences are not so great as to suggest that kicking in the dissected preparation and jumping have separate underlying motor programs. Indeed in view of the fact that dissected

locusts most commonly kick with both legs simultaneously, as if performing a jump (Fig. 2.1B), it was felt that the motor program studied in the dissected preparation does represent the program for jumping in the intact animal.

One aspect of the motor program which has not been reported previously is that FETi often has two distinct phases of activity during co-contraction. This is evident in EMG records (Fig. 2.1) but is especially clear in intracellular recordings from FETi (Fig. 2.2B,C). The first phase is a weak tonic excitation which lasts throughout most of co-contraction, and during which FETi fires at a quite constant frequency of 10-40 spikes/s. The second and concluding phase of FETi's activity is marked by a stronger pulse-like excitation causing the motoneuron to discharge at a frequency of 70-130 spikes/s.

The important question arising from these studies of the motor programs for jumping and kicking is how the patterns of activity seen in the flexor and extensor tibiae motoneurons are generated. The two phases of FETi's activity during co-contraction very likely have separate origins: the tonic excitation probably stemming from peripheral reflex mechanisms (Heitler and Burrows 1977b) and the final phasic excitation arising from a central neuronal system with bilateral outputs. The latter is suggested by the fact that the final burst in FETi very often commences simultaneously in both legs (Fig. 2.1B), and because the phasic excitatory input can still be seen in a leg which is prevented from kicking and cannot therefore be sending relevant proprioceptive information to the CNS (Fig. 2.5). The exact origin of this central input to FETi is unknown. Because the rapid FETi burst persists after the meso/metathoracic connectives have been cut and, in separate experiments, after the abdominal connectives have been severed, the neurons necessary for generating it must be restricted to the metathoracic ganglionic mass. This also excludes the C-neurons as the main source of the phasic input. Furthermore, the M-neurons, or whatever produces their depolarization, cannot be implicated because they sometimes discharge after the rapid burst in FETi has occurred (Fig. 2.4B). The interneurons described in Fig. 2.8 might contribute to the depolarization of FETi. They discharge at an appropriate phase and also possess branches

which terminate in the lateral neuropile in the region where branches of FETi occur. It is probable that the local neuron shown in Fig. 2.9 is also involved. Spikes in this cell are known to excite FETi and during a kick it also discharges at an appropriate phase. It is possible that the proprioceptive input which builds up during the initial stages of the motor program excites this hypothetical central system of interneurons, which in turn delivers a phasic excitation to both FETi motoneurons. Because successful kicks do occur in which the rapid FETi burst is lacking, the neuronal system responsible for the final extensor excitation cannot be an integral part of the kick trigger system itself, as the latter must still be producing its trigger pulse. Obviously, the FETi burst is not necessary for kicking or jumping to occur. When it does occur it probably ensures the maximum development of tension in the hind femora immediately prior to a jump and consequently increased force output during take-off.

The excitation which depolarizes the flexor motoneurons and initiates the entire motor sequence for kicking comes from an as yet unknown central source. Although the role of abdominal (and other) receptors excited by tactile stimuli in producing the flexor depolarization cannot be ruled out for every case, there were clear examples in which this depolarization occurred many seconds after the tactile stimulation of the body had ceased, i.e. there was no strict temporal association between the time of touching the insect's body and the onset of the flexor excitation. There were also times when a locust kicked several times in response to the one tactile stimulus and here the flexor motoneuron displayed a strong excitation during each kick. Once co-contraction is underway, however, sensory feedback from the hindlegs probably maintains the depolarization of the flexors (Heitler and Burrows 1977b).

The importance of the central FETi-flexor connection in generating the pattern of activity in flexors is probably minor. As can be seen in Fig. 2.3, the membrane potential of each flexor motoneuron is already close to its maximal depolarization before FETi even becomes active. This central connection adds a little extra excitation to the flexors early in co-contraction, but as the motor program continues its strength and importance rapidly

diminish.

In all recordings from flexor motoneurons the timing of the trigger inhibition which terminates flexor activity corresponds to the time at which the M-neurons become active. Although simultaneous recordings have not yet been made from an M-neuron and a flexor during a kick, all the available evidence continues to support the proposal that the M-neurons act as trigger interneurons for kicking and jumping. This is not to say, however, that the M-neurons are the only source of the inhibitory input to flexor motoneurons. For example, the interneuron shown in Fig. 2.7C appears to be the mesothoracic homologue of the M-neurons and has the appropriate discharge pattern to produce flexor inhibition.

One aspect of the jump motor program not observed in the preparation used in this study was the C-neuron's proposed synchronous activation of FETi and the flexor motoneurons at the beginning of co-contraction to lock the hind tibiae into full flexion (Pearson and Robertson 1981). Unfortunately, this response never occurs in dissected animals and so it was not possible to test this proposal during the present intracellular investigation. The C-neurons, however, play no part in maintaining the simultaneous activity of the flexor and extensor muscles during the first phase of co-contraction (Fig. 2.6). Instead, they probably provide a general excitation to these muscles towards the end of co-contraction and so generate further tension in the hindlegs.

Triggering of the Jump

It has been proposed that in order for the M-neurons to become activated and trigger the jump they must receive feedback from leg proprioceptors during the co-contraction phase (Steeves and Pearson 1982). This input is postulated to bring the membrane potential of the M-neurons closer to threshold, thereby activating them directly or enabling them to discharge in response to auditory or visual stimuli. If this proposal were true, the M-neurons should display a gradual depolarization during co-contraction leading to their rapid discharge. Yet they do not. Instead they exhibit a maintained hyperpolarization which is terminated by a very

sudden and strong depolarization (Fig. 2.4B-D).

This argues against the proposal that proprioceptive signals increase the excitability of the M-neurons during co-contraction and instead suggests that the excitatory input to each M-neuron arises from the sudden activation of a system of interneurons within the CNS. This system is referred to as the trigger system. The notion of a trigger system exciting the M-neurons is supported to some extent by the discovery of other interneurons which discharge near the end of the co-contraction phase (Figs. 2.7-9), although their activity may serve a different function such as generating the final phase of FETi's excitation (Fig. 2.9). The trigger system, and other neurons necessary to generate the motor program, must be located within the metathoracic ganglionic mass because the main features of the motor program can be produced after severing the meso/metathoracic connectives or the abdominal connectives. The neurons in the mesothoracic ganglion, whose activity suggested they were involved in generating the kick, must either play a minor, supporting role in producing the motor program or play another role, such as providing input to the flight system before a jump or generating appropriate movements in the pro- and mesothoracic legs prior to take-off.

That the timing of the M-neuron discharge falls under central rather then peripheral control would allow more precise control of motor events. The maintained hyperpolarization of the M-neurons throughout co-contraction ensures these cells remain inactive and thus prevents them from inhibiting the flexor motoneurons until sufficient tension has developed in the legs. Furthermore, the rapid, strong depolarization of both M-neurons would ensure the synchronous inhibition of the flexor motoneurons of both hindlegs and thus the synchronous timing of leg extension during jumping. This synchrony would be more difficult to achieve if M-neuron activation depended on a gradual depolarization by proprioceptive input during the co-contraction phase. This is because synchronous activation would require the membrane potential of each M-neuron to be in an identical state with respect to spike threshold and each to receive identical synaptic input.

Although the M-neurons, based on their known connections and discharge pattern, definitely inhibit the flexors and so contribute to triggering the kick or jump, it has not yet been possible to establish whether they are necessary for triggering. Attempts to hyperpolarize an M-neuron, thereby preventing its burst of activity during kicking, have failed because of an inability to deliver enough negative current to the cell to silence its discharge. Since there are a number of other interneurons which burst at the time the M-neurons do (Figs. 2.7C, 2.8A,C) it is possible that M acts in concert with some of these to produce the trigger inhibition in the flexors.

In summary, the data indicate that an important event in triggering the jump is the strong, pulse-like depolarization of the M-neurons at the end of co-contraction. It is proposed that an interneuronal system (the trigger system) is responsible for the generation of this input. The future direction of studies of the neuronal mechanisms for jumping must now be to determine the cellular connections between neurons of this trigger system.

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III. AN EVALUATION OF THE ROLE OF IDENTIFIED INTERNEURONS IN TRIGGERING KICKS AND JUMPS[2]

A. INTRODUCTION

When studying the neuronal system responsible for generating a particular behavior, any information about the importance of an individual cell can help to shed light on the organization and functioning of the system as a whole. Consequently, the question of how important single cells are for the performance of various behaviors has been the subject of many studies. Most of these have focused on determining what role identified interneurons play in the initiation of rhythmic movements or escape responses (e.g. Eaton and Bombardieri 1978; Gillette et al. 1978; Weeks and Kristan 1978; Wine and Krasne 1982; McCrohan 1984; Nolen and Hoy 1984; Pearson et al. 1985), with the particular role of a neuron being assessed in terms of its sufficiency and necessity for generating the behavior under examination. In exceptional cases individual cells can be of critical importance, the best example being that of the lateral giant fibers of the crayfish. These interneurons are both necessary and sufficient for the generation of the short latency tailflip in response to mechanical stimulation of the animal's abdomen (Wine and Krasne 1982). More typically, however, single cells are found which, although sufficient to evoke a particular behavioral response, are not always necessary for the response to occur. This may be because behavioral initiation is the responsibility of a small group or population of these cells, all of similar nature and acting in concert, such that the importance of any one cell in the group is reduced. Examples of systems of neurons organized in this way include the paracerebral neurons which initiate the feeding rhythm in the marine gastropod mollusc *Pleurobranchaea* (Gillette et al. 1978) and the 404 interneurons which evoke flight in the locust (Pearson et al. 1985). Alternatively, the lack of necessity of a single cell may be because there are multiple or parallel pathways by which the behavior can

[2]A version of this chapter has been accepted for publication. Gynther IC, Pearson KG (1988) J Neurophysiol

be elicited, as in the case of interneuron 204 and interneurons 21 and 61 in the leech swimming system (Weeks and Kristan 1978; Nusbaum and Kristan 1986), the slow oscillator interneuron and the cerebral ventral cells in the feeding system of the pond snail (Rose and Benjamin 1981; McCrohan 1984) and Mauthner and non-Mauthner evoked fast-starts in teleost fish (Kimmel et al. 1982; Eaton 1983, 1986). The present investigation examines the importance of particular identified interneurons for initiating movement in the neuronal system underlying another well-studied escape response, the locust jump.

Although jumping and kicking in Orthopteran insects such as locusts involve highly ballistic movements, the motor program which produces both these behaviors spans a period of about half a second prior to movement onset. This program is comprised of three distinct phases (Heitler and Burrrows 1977a). Firstly, the tibiae of both hindlegs are flexed so as to adopt the correct position for jumping or kicking. Secondly, there is a prolonged co-contraction of the flexor and extensor muscles in the hind femora during which time the weaker flexor muscle is able to hold the leg flexed by virtue of its mechanical advantage over the powerful extensor muscle (Heitler 1974). Consequently, the extensor undergoes an isometric contraction and the large amount of force thus generated causes a marked deformation of elastic cuticle of the femoral-tibial joint. Most of the energy for the jump or kick is stored as this elastic deformation. The final phase of the motor program, which is responsible for triggering the actual movement, is a sudden cessation of all flexor muscle activity. This allows the extensor muscle to shorten and the energy stored in the cuticle to be rapidly released, enabling the tibiae to extend explosively.

At present the neuronal mechanisms involved in the initiation and maintenance of the co-contraction phase are partially understood (Heitler and Burrows 1977b; Pearson and Robertson 1981) but there is no direct evidence about the way in which the program's final phase - the termination of flexor activity - is brought about. Three conceivable mechanisms by which this might occur are : 1) by an inhibition of the flexor motoneurons, 2) by a cessation of excitatory input to the flexor motoneurons, or 3) by a combination of these two

events. Of these ideas, only the first has received consideration due to the discovery of a pair of metathoracic interneurons, called M-neurons, which monosynaptically inhibit hindleg flexor motoneurons and display many features which suggest they might be responsible for triggering kicks and jumps (Pearson et al. 1980; Steeves and Pearson 1982). Further support for the involvement of the M-neurons came from the finding that they discharge high frequency bursts at the end of the motor program (Chapter 2). Other evidence presented in that chapter, however, indicates that the activity of the M-neurons may actually be controlled by a higher order system within the CNS and that they may not be decision-making elements of the circuitry for triggering kicks and jumps as had been thought. In addition, several other interneurons were found to discharge at the same time as M during the motor program. In the light of this new evidence it would now seem necessary to determine whether the M-neurons are indeed a crucial part of the jump system and whether they, alone, are responsible for terminating activity in the flexors when a jump is triggered.

Here these questions have been examined directly by testing the sufficiency and necessity of the M-neurons for triggering bilateral kicks, a behavior with a motor program very similar to jumping. It was found that although the M-neurons are able to trigger kicks, this apparent sufficiency is not physiological since it requires that higher than natural spike frequencies be experimentally-evoked in the neuron. Also, abolishing normal spike activity in the M-neurons by hyperpolarization does not prevent bilateral kicking. These results indicate that the M-neurons are not a necessary component of the neuronal system and that other cells must be involved in terminating the activity of flexor motoneurons at the end of the motor program. One such interneuron has been identified but it may function by inhibiting the neurons which excite flexor motoneurons during co-contraction rather than by inhibiting the flexors directly. The way in which these findings have altered the existing view of the neuronal organization of the system for triggering kicks and jumps will be discussed.

B. MATERIALS AND METHODS

Preparation of Animals

Adult male *Locusta migratoria* from a long-established colony at the University of Alberta were used in all experiments. The preparation which enabled intracellular recording from the thoracic CNS during bilateral kicks of the hindlegs was described in Chapter 2. This preparation is shown diagrammatically in Fig. 3.1A. Electromyographic recordings of the hindleg flexor and extensor tibiae muscles were obtained by inserting two 200 µm copper wire electrodes (insulated except for their tips) into the cuticle of the femora. This provided a combined recording of flexor and extensor EMGs in which the activity of both muscles could be clearly distinguished based on the amplitude of their spikes. Activity of the extensor muscle is evident as large amplitude potentials whereas flexor muscle activity is characteristically of much smaller amplitude (Fig. 3.1B). Approximately 80% of locusts prepared in this way performed bilateral kicks of the hindlegs in response to tactile stimulation of the abdomen with a pair of forceps.

In some experiments, the timing of the rapid tibial extension which occurs during a kick was monitored using an optical switch (Texas Instruments integrated circuit no. 8519), positioned such that its infrared light beam was interrupted only when the tibia was fully flexed (Fig. 3.1). For simplicity, this optical switch is referred to as a photocell throughout the chapter.

Intracellular Recording and Dye-filling

Intracellular recordings from interneurons and motoneurons were made using glass microelectrodes filled with either 1M potassium acetate or a 5% aqueous solution of the dye Lucifer Yellow. Resistances of these electrodes were typically between 30 and 80 megohms. Penetrations of flexor motoneurons and M-neurons could be recognized solely by physiological criteria. In those cases, however, in which it was necessary to determine which member of the

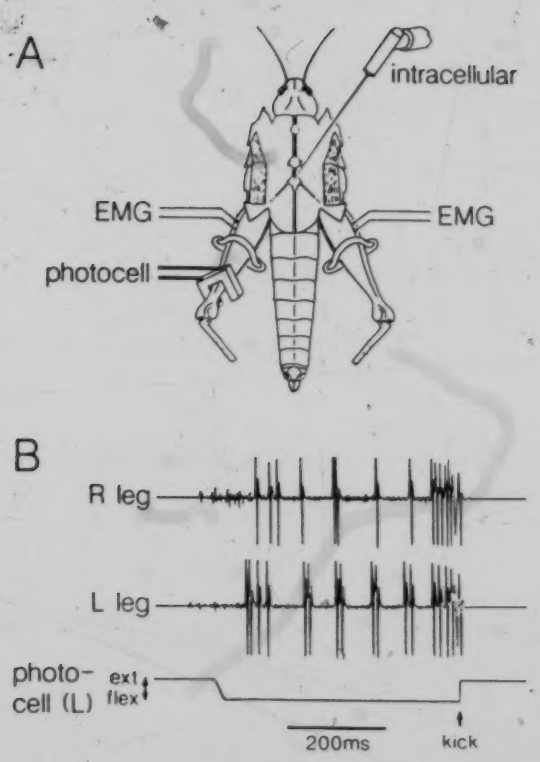

Fig. 3.1. Preparation used to study bilateral kicking, and a typical example of the motor program for this behavior. A Diagram of the experimental setup for intracellular recording during kicks. The photocell was placed so as to monitor movement of the tibial stump into or out of the fully flexed position. B Electromyographic recordings from left and right legs, and a photocell recording of the left leg's position during a bilateral kick. A downward deflection of the photocell trace represents movement of the tibia into full flexion; an upward deflection signals extension out of this position. During a kick, each leg was initially flexed and then remained so while the flexor and extensor muscles co-contracted. The kick was triggered when flexor activity suddenly ceased (not visible in EMG records but see photocell trace). Although the time of tibial extension is shown for the left leg only, both legs extended almost synchronously, as is evident from the close matching of left and right leg EMG patterns.

bilaterally symmetrical pair a particular M-neuron was, or when a different interneuron had been penetrated, Lucifer Yellow was injected following the recording by applying a constant hyperpolarizing current of 5 nA for at least 5 minutes. After injection the ganglia were removed and fixed in 4% paraformaldehyde for 30 minutes. They were then dehydrated through an ascending series of alcohols, cleared in methyl salicylate and viewed as wholemounts under an epifluorescence microscope.

All recordings were stored on magnetic tape and later displayed on either a Gould ES1000 chart recorder or a Gould 2400S pen recorder. Measurements of various latencies and spike frequencies were made with a digital oscilloscope.

Timing of Pulses During Co-contraction

Some experiments involved injecting interneurons with intracellular current pulses during the co-contraction phase. Because of the short duration of the co-contraction period it would have been impossible to time the delivery of these pulses manually. Consequently, a digital device was used to to produce an accurately timed output pulse. This device counted extensor muscle spikes (standardized as pulses from a window discriminator) and whenever a specified number of spikes occurred within a designated duration an output pulse was produced. This pulse in turn was used to trigger a stimulator. During these pulse injection experiments it was necessary to use depolarizing currents of as much as +30 nA because the neurons being injected had very high thresholds and were simultaneously receiving hyperpolarizing synaptic input. Also, large currents had to be used in order to evoke the desired high spike frequencies (>400 spikes/s). Hyperpolarizing current pulses of up to -20 nA were required to suppress all spike activity in the M-neurons at the time that these cells received their strong excitatory synaptic input. Currents of these magnitudes made balancing the bridge for the intracellular electrode a difficult, and sometimes impossible, task.

C. RESULTS

The M-neurons are a mirror image pair of interneurons which make powerful inhibitory connections with flexor motoneurons on opposite sides of the metathoracic ganglion (Pearson et al. 1980). Each M-neuron inhibits the flexors of one hindleg only. It was shown in Chapter 2 that during bilateral kicks in response to tactile stimulation of the abdomen the timing of the high frequency discharge in the M-neurons occurs at a phase of the motor program (measured relative to flexor and extensor muscle activity) that suggested these neurons function to terminate flexor activity and thereby trigger the kick. However, it is not possible from EMG recordings alone to determine the exact time at which the trigger inhibition causes flexor muscle activity to cease because the small flexor potentials are not visible amidst the final burst of large amplitude extensor spikes (see Fig. 3.1B). Therefore, in order to establish unequivocally that M's burst occurs at the correct phase for it to directly contribute to the process of triggering, it was necessary to record simultaneously from an M-neuron and an ipsilateral flexor motoneuron. Such a recording is shown in Fig. 3.2. It can be seen that the onset of the rapid repolarization of this flexor motoneuron at the end of the motor program coincided precisely with the first spike in the M-neuron's discharge, confirming that M must be involved in triggering the kick. Moreover, no IPSPs were apparent in the flexor other than those that correspond one for one with spikes in the M-neuron, suggesting that M might be the only interneuron responsible for flexor inhibition. However, such a strict temporal relationship between the onset of M-neuron activity and flexor repolarization was not always evident. In 38% of the 90 kicks elicited from 33 animals, the flexor motoneuron began to repolarize *prior* to the first spike in the M-neuron. The rate of this repolarization was usually much slower than occurs once the M-neuron discharge has commenced. Fig. 3.3 illustrates one of the most extreme examples of this phenomenon. The gradual nature of this repolarization and the absence of any obvious IPSPs in the flexor during its course suggested that it may have been due to a decrease in flexor excitation rather than a direct inhibitory input. However, the possibility that it was caused by inhibition from

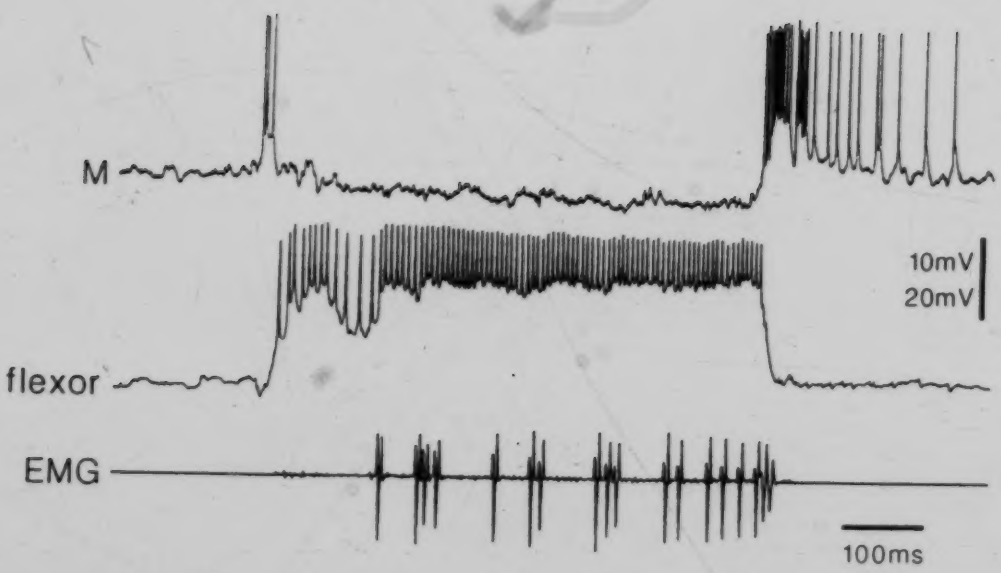

Fig. 3.2. Precise correspondence between the timing of the M-neuron's discharge and the onset of flexor inhibition during a bilateral kick. Top trace - intracellular recording from an M-neuron; middle trace - intracellular recording from a flexor motoneuron that received inhibitory input from this M-neuron; bottom trace - flexor and extensor EMGs from the leg innervated by the flexor motoneuron. Note that the first spike in M's high frequency burst exactly coincided with the sudden repolarization of the flexor motoneuron. Individual IPSPs in the flexor, which correspond one for one with spikes in the M-neuron, are visible following the kick. No other inhibitory potentials are apparent in the motoneuron.

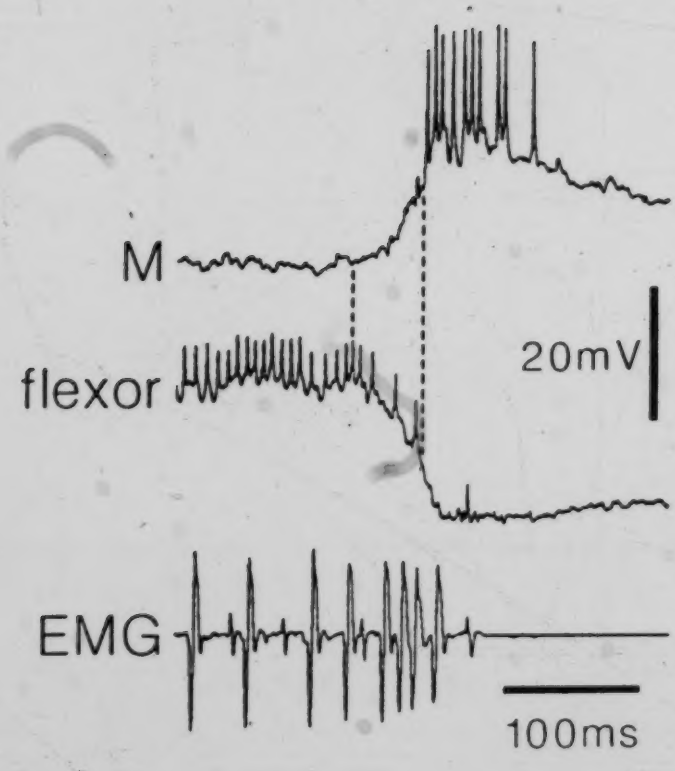

Fig. 3.3. Flexor repolarization commencing before the discharge in the M-neuron. Top trace - intracellular recording from an M-neuron; middle trace - intracellular recording from an ipsilateral flexor motoneuron; bottom trace - EMG recording from the leg innervated by the motoneuron. Only the final part of the motor program is shown. Note that there was a slow repolarization of the flexor's membrane potential prior to the first spike in the M-neuron yet coincident with M's ramp depolarization. In this particular example the M-neuron discharged quite weakly, however these slow flexor repolarizations were also observed preceding powerful M-neuron bursts.

nonspiking interneurons cannot be ruled out. It was also noted that the onset of the slow repolarization nearly always corresponded to the start of the ramp depolarization which precedes the burst of spikes in the M-neuron. Occasionally the flexor's repolarization began after the onset of M's ramp, but never did it precede this depolarization. Consequently, the durations of these two events were closely correlated. When the ramp depolarization in M was very sudden, as in Fig. 3.2, there was no obvious sign of repolarization in the flexor prior to the first spike in the interneuron. Only when M's ramp was more prolonged, as in Fig. 3.3, did the slow repolarization of the flexor become apparent.

Further evidence that the M-neurons play a direct role in the triggering process is that a close temporal relationship existed between the onset of spike activity in an M-neuron and the start of tibial extension during a kick. By using a photocell to monitor when the tibia moved into or out of the fully flexed position, it was found that the first spike in an M-neuron burst always preceded the onset of extension of the ipsilateral tibia by, on average, 32 ms (S.D.=8.8, n=51). This value is actually a slight overestimation because a small amount of tibial extension had to occur before any movement was registered by the photocell. Although the example in Fig. 3.4 shows the timing of extension of the left leg only, it should be noted that the left and right legs extend in near synchrony during normal bilateral kicks. This usual timing of hindleg extension relative to the pattern of EMG activity will become important for purposes of comparison with results presented in the following sections.

Are the M-neurons Sufficient to Trigger Kicks?

The above findings raised questions concerning the M-neurons' importance in the triggering process, i.e. are these neurons necessary and sufficient for triggering kicks and jumps? To address the sufficiency question, one member of the pair of M-neurons was injected with a brief pulse of depolarizing current during the co-contraction period, at a time when it would not normally have been active (Fig. 3.5). The high frequency discharge evoked by the pulse caused hindleg flexor and extensor EMG activity to cease abruptly on the side to

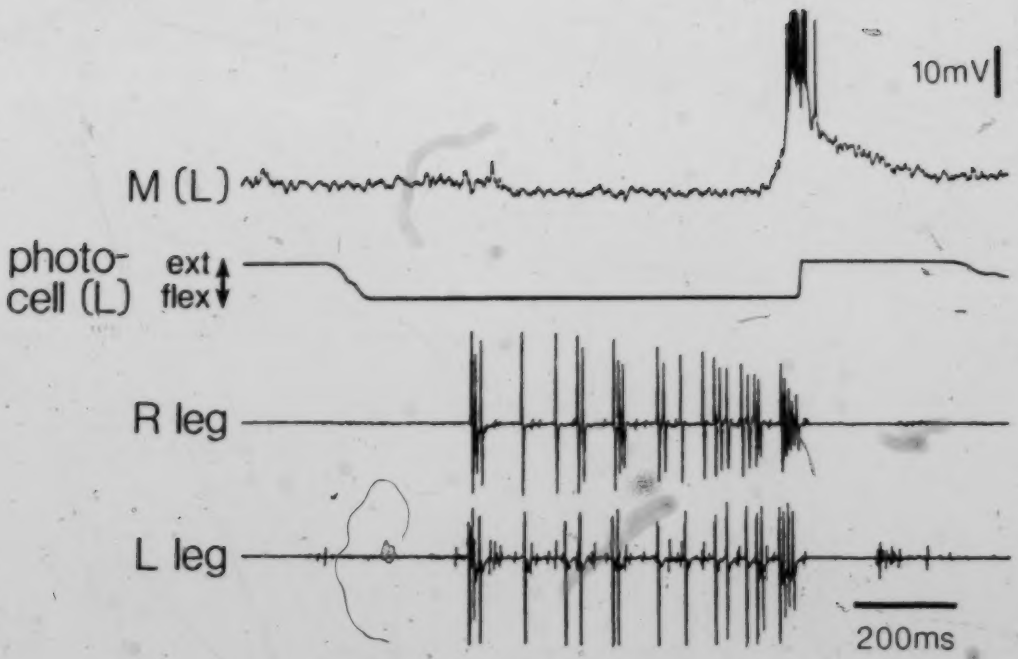

Fig. 3.4. Timing of the M-neuron's discharge relative to the start of tibial extension during a bilateral kick. Top trace - intracellular recording from the M-neuron which inhibited left leg flexors; second trace - photocell record of the position of the left tibia during the motor program; bottom traces - EMGs from the left and right legs. Note that the first spike in M's burst preceded the onset of the left leg's extension (as judged by the sudden upward deflection of the photocell trace) by approximately 30 ms.

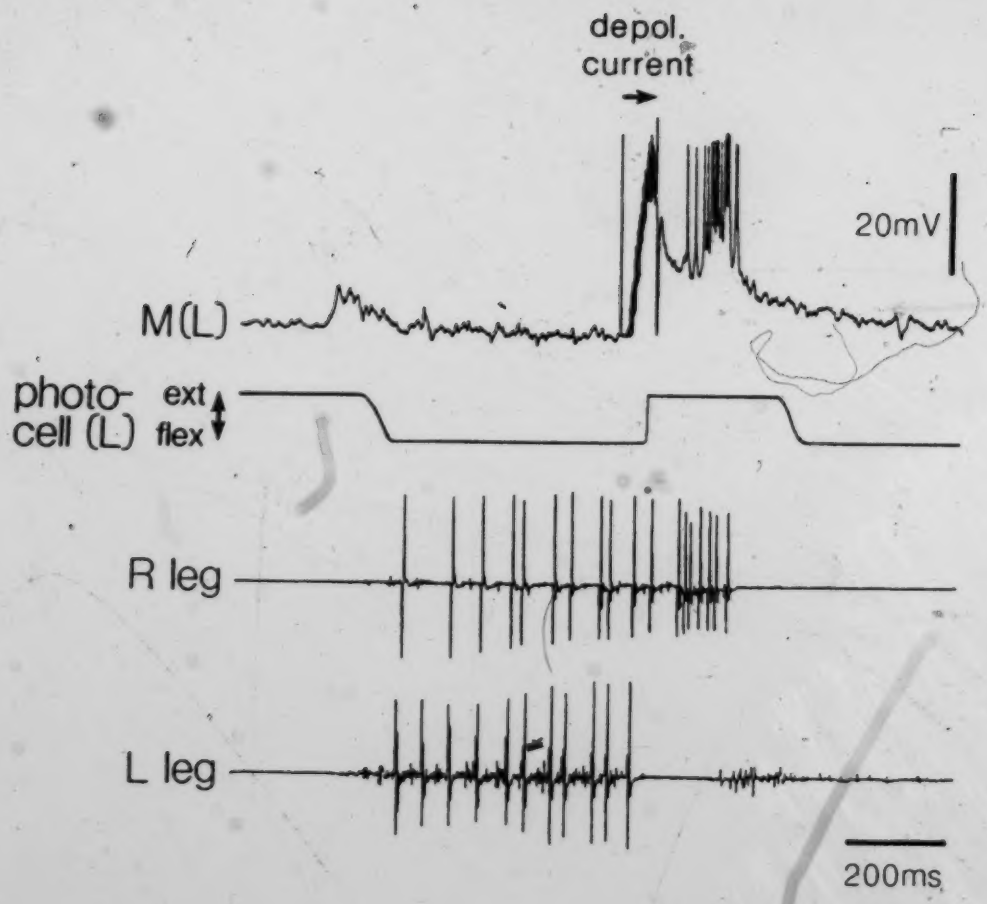

Fig. 3.5. Premature triggering of leg extension produced by strongly depolarizing an M-neuron. Top trace - intracellular recording from the M-neuron which inhibited left leg flexors; second trace - photocell monitor of the left leg's position; bottom traces - left and right leg EMGs. When the M-neuron was injected with a brief pulse of depolarizing current during the co-contraction period, EMG activity in the left leg (i.e. on the side of M's axonal branch) abruptly ceased, and this leg extended prematurely. Note the time of extension of the left leg (see photocell deflection) relative to the EMG activity in the right leg, which continued unaffected.

which this M-neuron's axonal branch projected and caused extension of this leg to be triggered prematurely (see photocell trace and compare with Fig. 3.4). The EMG pattern in the opposite leg, however, was unaffected by the current pulse and the motor program in this leg continued on as normal. This is very clear in Fig. 3.6 in which the photocell monitored movement of the leg on the side opposite to the recorded M-neuron. This leg kicked at the normal time (cf. Fig. 3.4) even though the current pulse in the M-neuron had caused the other leg to extend much earlier. Thus the effect of injecting a strong depolarizing pulse into one M-neuron was to produce a very noticeable discrepancy in the timing of left and right leg extension, something that was never observed during normal bilateral kicks.

This result also confirms the finding of Pearson et al. (1980), that there is no coupling between the two M-neurons, otherwise a depolarizing pulse in one neuron would cause early extension of both legs. This was further demonstrated by injecting depolarizing current into one M-neuron during a bilateral kick while simultaneously recording from the other M-neuron (Fig. 3.7). The burst of spikes evoked in the M-neuron projecting to the right side flexors caused early triggering of the right leg yet failed to elicit spikes in the left M-neuron. Consequently, the left leg kicked at the usual time. A single action potential was evoked in the left M-neuron but it occurred after the depolarizing pulse had ceased and was probably due to a reflex input caused by extension of the right leg. A corresponding subthreshold excitatory input was apparent in the right M-neuron. These depolarizations were never seen following pulses that failed to cause leg extension.

An obvious feature of these current injection experiments was that even when a pulse in an M-neuron triggered the early extension of one hindleg, this same neuron discharged again, although more weakly, at the time that it would normally have become active (Figs. 3.5-7). In Chapter 2 a similar result was noted in that an M-neuron still fired a weak burst of spikes at the correct phase of the motor program even if the corresponding leg was prevented from kicking altogether. Interestingly, it can be seen in Fig. 3.7B that after the depolarizing pulse had caused co-contraction to cease in one leg, the strength of the subsequent burst in

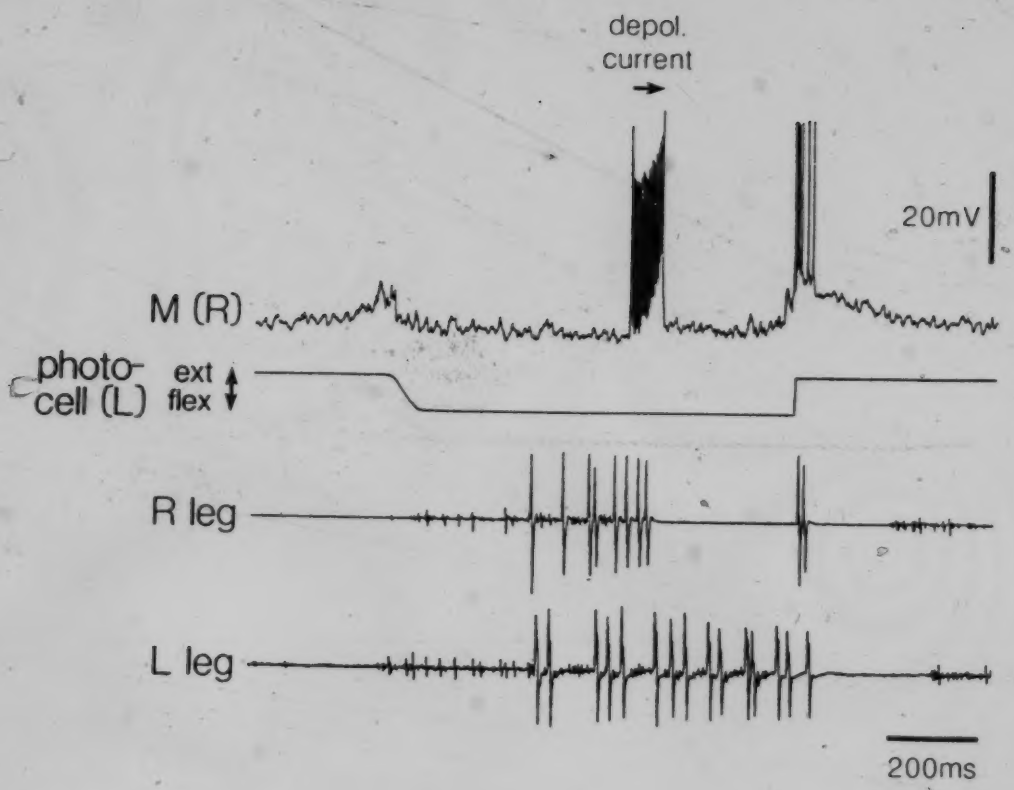

Fig. 3.6. Premature triggering of one leg does not affect the time of extension of the opposite leg. Top trace - intracellular recording from the M-neuron that inhibited flexors on the right side of the metathoracic ganglion; second trace - photocell record of the position of the left tibia; bottom traces - EMGs from the left and right legs. Evoking a burst of activity in the right M-neuron by applying a depolarizing current pulse caused early triggering of the right leg (see sudden cessation of EMG) but had no effect on the left leg, which continued with its motor program and extended at the usual time.

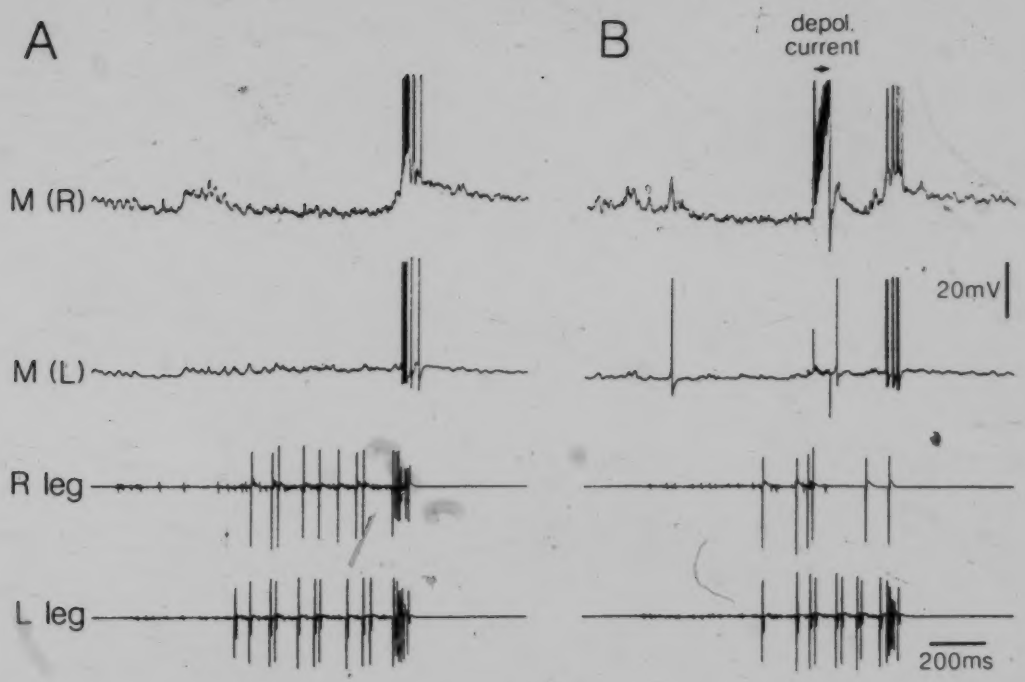

Fig. 3.7. Lack of direct coupling between the two M-neurons during a bilateral kick. Top traces - intracellular recordings from the M-neuron which inhibited right leg flexors. This cell was penetrated in its medial processes. Second traces - intracellular recording from the opposite M-neuron, i.e. the one inhibiting left side flexors. This recording was made laterally in the neuron's large axonal branch. Bottom traces - EMGs from the left and right legs. **A** Normal pattern of activity in both M-neurons during a bilateral kick. **B** Injection of a depolarizing current pulse into the right M-neuron during co-contraction triggered right leg extension (see cessation of usual EMG pattern) but failed to evoke any spikes in the left M-neuron. Consequently, the motor program in the left leg continued as normal. The single spike in the left M-neuron following the pulse was probably due to a reflex invoked by extension of the right leg. Note that after the pulse both M-neurons discharged more weakly than they did during the normal bilateral kick (compare strength of bursts in **A** and **B**).

the opposite M-neuron was also weaker than normal.

The results of these experiments suggest that the M-neurons are sufficient to trigger kicks. However a neuron should only be considered to be sufficient when the level of activity required to evoke the behavior artificially is comparable to that occurring during the natural performance of the behavior. This was not so for the M-neurons. In eight animals a comparison was made between the highest discharge rate seen in an M-neuron during normal kicks and the lowest discharge rate evoked in the same neuron when a kick was triggered by current injection. The mean of the peak frequencies during normal kicks was 373 spikes/s (S.D.=27.7). This was well below the mean value of 438 spikes/s (S.D.=44.4) required for kicks to be triggered artificially. Since I was not able in all animals to deliver a series of depolarizing pulses in which the evoked M-neuron frequency increased in gradual steps, some of the values for the lowest pulse-evoked frequencies were probably higher than would actually have been necessary to trigger extension. However, in no individual was it possible to elicit a kick artificially unless the M-neuron discharge rate was greater than the highest natural rate seen in that neuron. This result demonstrates that physiological levels of activity in the M-neurons are not sufficient to trigger kicks and therefore supports the earlier finding that the normal process by which flexor motoneuron activity is terminated does not depend on the M-neurons alone.

There were also several behavioral indications that some aspect of the triggering process in the artificially evoked kicks was abnormal. The most striking was that even for those depolarizing pulses which were able to trigger leg extension, the latency between the onset of the pulse and the start of tibial extension was much longer (mean=70 ms, S.D.=16.9, n=33) than the corresponding latency of 32 ms between the onset of a natural M burst and tibial extension (see earlier data; Student's t-test, $p<0.001$). In addition, the hindleg extension produced artificially was never accompanied by the audible click which is normally characteristic of this behavior. The click is the result of a mechanical lock (associated with a specialization of the flexor tendon) being overcome at the moment of

triggering (Heitler 1974). It is not likely that these unusual features of pulse-evoked kicks were caused by insufficient force being generated in the femur as a result of the premature interruption of co-contraction because they occurred even when the pulse came very late in the co-contraction phase, at a time when considerable force would already have developed. Instead, they may have been caused by the pulse in the M-neuron failing to produce an immediate and synchronous inhibition of all the flexor motoneurons. Any flexor spikes that occurred after the onset of the M pulse (e.g. see Fig. 3.8) would tend to counteract the fall in tension in the flexor muscle and so retard leg extension. Another possible explanation as to why the artificially triggered extension may have been delayed is because the inhibitory motoneurons to the flexor muscle presumably would not have been activated. According to Heitler and Burrows (1977a), these motoneurons fire a burst of spikes just prior to the moment a kick is triggered and thereby speed relaxation of the flexor muscle. Without their usual discharge there may have been some residual tension in the flexor muscle which would also have opposed extension of the tibia during the kick.

The indications from the preceding results that more than just M-neuron inhibition of flexors is required to terminate activity in these motoneurons during normal kicks and jumps were verified by observations of the nature of flexor motoneuron repolarization during kicks evoked by depolarizing pulses. Figure 3.8 shows a simultaneous recording of an M-neuron and an ipsilateral flexor motoneuron during a kick in which depolarizing current was injected into M. Because the electrode was not balanced it is not possible to determine what discharge frequency was evoked in the M-neuron but clearly it was enough to shut down almost all flexor activity (see EMG trace) and cause premature extension of the appropriate leg. However, the flexor inhibition produced during the depolarizing pulse was unlike that associated with M's activity during natural kicks (e.g. Figs. 3.2, 3.3) in that it did not return the flexor's membrane potential to its resting level. This is especially clear in Fig. 3.9 in which examples of flexor inhibition during naturally and artificially-evoked kicks from the same animal have been superimposed. Although the rate of inhibition at the onset of the

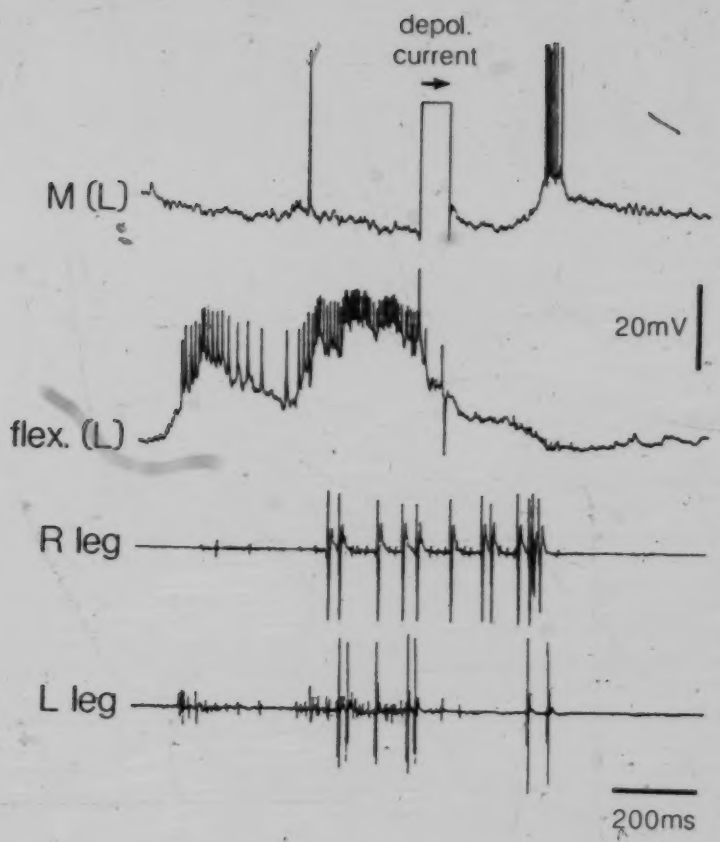

Fig. 3.8. Incomplete flexor repolarization during a kick evoked by a depolarizing pulse in M. Top trace - intracellular recording from the M-neuron which made inhibitory connections with left side flexors; second trace - intracellular recording from a left leg flexor motoneuron; bottom traces - left and right leg EMGs. The depolarizing curent pulse in the M-neuron during co-contraction terminated flexor activity in the left leg and caused this leg to extend prematurely. Note, however, that the pulse was not able to produce a complete repolarization of the flexor motoneuron.

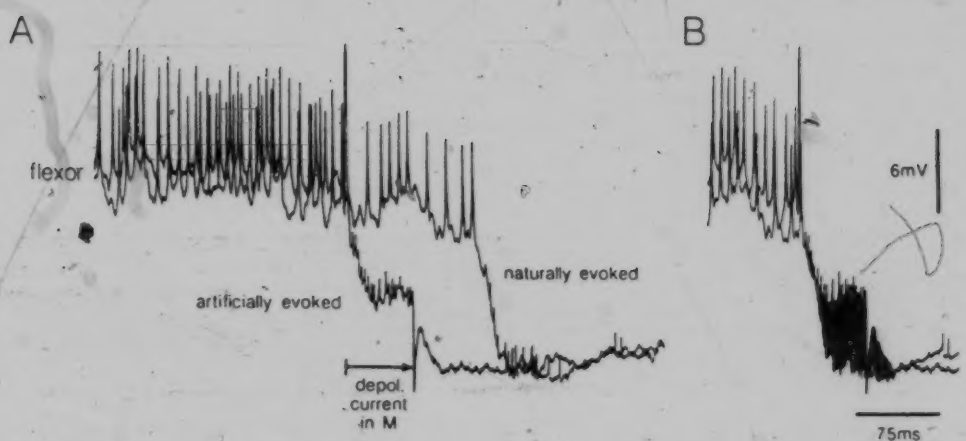

Fig. 3.9. Differences in the nature of flexor inhibition during natural and pulse-evoked kicks. Two intracellular traces from the same flexor motoneuron, one during a natural bilateral kick and one during a kick evoked by a depolarizing pulse in the ipsilateral M-neuron (see arrow in A), have been superimposed. A The two traces were aligned relative to the last extensor spike in the EMG of the opposite leg (not shown). B The same traces were aligned relative to the onset of the flexor's inhibition in order to emphasize the difference in the rates of repolarization. Note that during the artificially-evoked kick, the inhibition of the flexor motoneuron was not complete and it displayed a brief plateau of depolarization. The tiny spikes in these recordings are due to capacitive pick-up of activity in the M-neuron.

depolarizing pulse was similar to that seen during the natural kick, the artificially-evoked activity in M did not cause a complete repolarization of the motoneuron. Instead, the flexor displayed a plateau-like depolarization which lasted for the remainder of the pulse. This effect was not due to a low discharge rate in the M-neuron because in this example the artificially-evoked frequency in M was 45% higher than M's frequency during the natural kick. Nor was it due to the flexor's membrane potential nearing the reversal potential for the IPSP because Fig. 3.10A shows that the same pulse in M could hyperpolarize this motoneuron to below its resting level. It is more likely that the plateau was the result of the inhibitory action of the M-neuron competing against a continuing excitatory drive to the flexor. Thus, the shape of the flexor repolarization produced by an M-neuron pulse midway through co-contraction may reflect a balance between excitatory and inhibitory influences on the motoneuron. At this stage of the motor program, the M-neuron may not be sufficient to trigger kicks when discharging at its natural frequency because it is competing against the neurons which provide excitation to the flexors. However, at the end of the motor program when M is normally active, this competition would not exist, providing the level of flexor excitation declined concurrently with the onset of M-neuron activity.

Taken together, the results of these experiments testing the sufficiency of the M-neurons argue strongly that the neuronal mechanism responsible for triggering kicks and jumps may involve not only a direct inhibition of flexor motoneurons but also a simultaneous cessation of flexor excitation.

Are the M-neurons Necessary for Triggering Kicks?

In the light of the above findings it was worthwhile to ask whether the contribution of the M-neurons is necessary for kicks to be triggered. In order to test this, M's normal burst of activity during a bilateral kick was abolished by injecting the cell with a prolonged hyperpolarizing current pulse (Fig. 3.11). The M-neuron received its usual depolarization but failed to generate any action potentials. Despite this, both legs kicked at the normal phase of

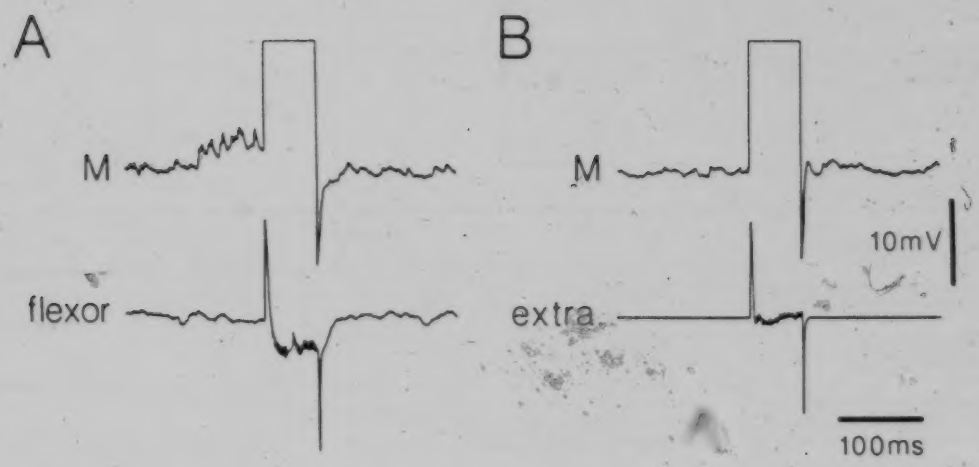

Fig. 3.10. Depolarizing current pulses in an M-neuron hyperpolarize ipsilateral flexor motoneurons. This is the same M-neuron/flexor pair that provided the example shown in Fig. 3.9. A The current pulse in the M-neuron (top trace) was delivered while the flexor motoneuron (bottom trace) was at rest. The burst of spikes evoked in M hyperpolarized the flexor 3 mV below its resting level. B To rule out the possibility that the effect seen in A was due to a direct electrical coupling between the electrodes, rather than a synaptic effect, the same pulse was delivered to the M-neuron after the flexor electrode had been withdrawn from the cell (extra). There was no evidence of any such coupling.

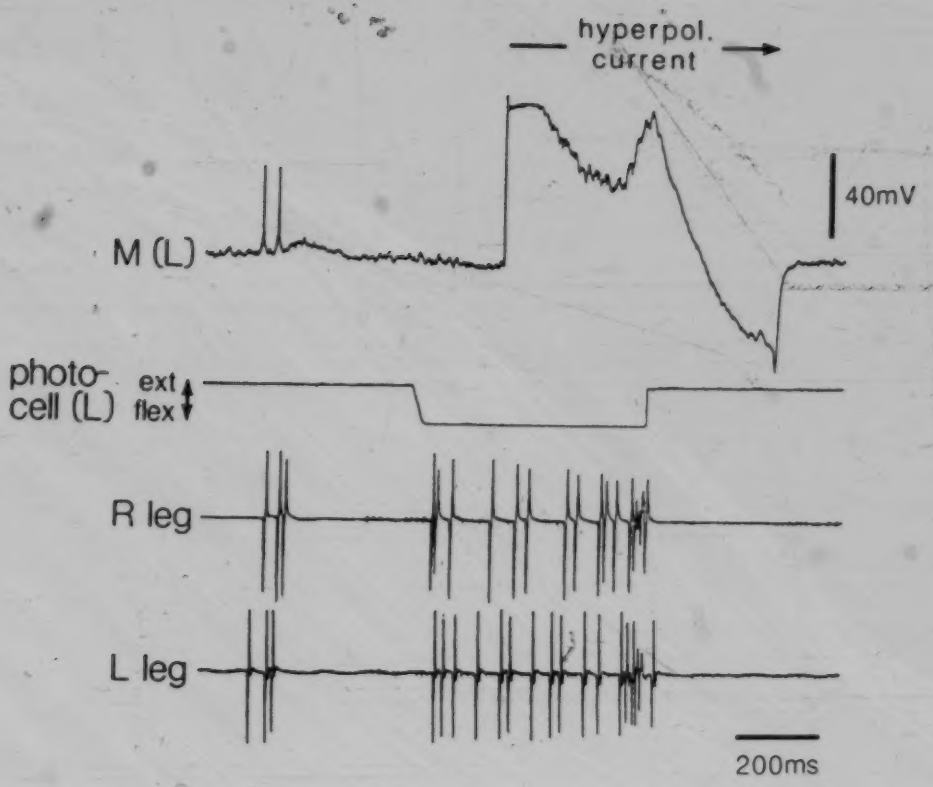

Fig. 3.11. Abolishing spike activity in an M-neuron does not prevent bilateral kicking. Top trace - intracellular recording from the M-neuron that inhibited left leg flexors; second trace - photocell monitor of the position of the left tibia; bottom traces - left and right leg EMGs. Suppressing this M-neuron's usual burst of spikes during a kick by applying a hyperpolarizing current pulse had no effect on either the EMG pattern or time of extension of the left leg, and both legs still kicked simultaneously. Note that the M-neuron still received its normal depolarizing input during the trigger phase.

the motor program (compare timing of photocell trace deflection with Fig. 3.4). The M-neurons, therefore, are not necessary for the triggering process.

For these kicks in which the M-neuron was hyperpolarized it was possible to gain an approximate value for the latency between the moment of triggering and the time of leg extension (monitored with the photocell) by using the shape of the rising phase of M's subthreshold depolarization to estimate when the first spike would have been expected to occur. In the one animal in which this latency was determined, the mean value was 32 ms (S.D.=3.9, n=7). This did not differ from the mean calculated for normal kicks (Student's t-test, $p>0.05$). Insofar as the latency to extension is a good indicator of behavioral performance, these results suggest that abolishing M-neuron activity has no apparent effect on the process by which bilateral kicks are triggered. Yet given the timing and intensity of the M-neuron's normal discharge and the powerful inhibitory influence of these neurons on flexors, it did not seem possible that preventing M's activity during kicks could be totally without effect. In an attempt to resolve this dilemma, the M-neuron hyperpolarization experiment was repeated in another animal while also recording from a flexor motoneuron on the appropriate side. The time of onset of repolarization in the flexor was not noticeably altered by the absence of M-neuron activity, although there was more variability in the rate of this repolarization from kick to kick than is normally seen. Also, it was noted that during kicks in which the M-neuron was active, the flexors always repolarized more quickly than when M's discharge was prevented (Fig. 3.12). For two normal kicks that were recorded, the times elapsed between the last spike in the flexor and the moment the motoneuron became fully repolarized were 24 and 26 ms. The corresponding values for four kicks in which the M-neuron burst was abolished ranged from 40 to 66 ms (mean=51.5 ms). Despite this slower and more variable repolarization, the latency to extension (as judged here by the timing of the mechanical artifact and subsequent spike in the flexor following repolarization) was no different from kicks in which the M-neurons were active. This was in agreement with our photocell measurements. Thus, the results from these two animals do not indicate that the

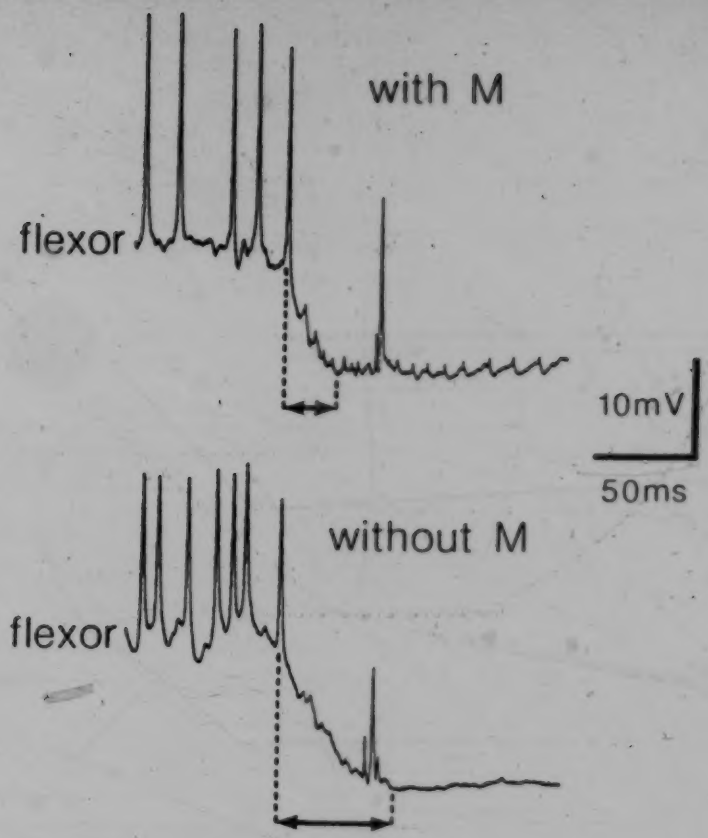

Fig. 3.12. The rate of flexor repolarization during a kick is slowed when M-neuron activity is suppressed. Intracellular recordings from a single flexor motoneuron during a normal kick (top trace) and during a kick in which activity in the ipsilateral M-neuron had been abolished by injecting hyperpolarizing current (bottom trace). Only the final portion of the flexor's activity during the motor program is shown. Note that when the M-neuron fired its normal burst of spikes the flexor repolarized more rapidly than when M's activity was prevented. The arrows indicate the time required for the membrane potential to return to resting level. Note also that no IPSPs are apparent in the flexor when spiking in the M-neuron had been suppressed. The artifact and single spike following the flexor's repolarization in each of these recordings was caused by the ballistic extension of the hindlegs. A comparison of the two traces shows that the timing of leg extension was not altered by abolishing the M-neuron's discharge.

M-neurons make a significant functional contribution to the triggering of bilateral kicks, however it could be misleading to draw any definite conclusions given the limited extent of this data (see Discussion).

In Fig. 3.12 the IPSPs in the flexor motoneuron caused by spikes in M are very evident. By contrast, when M-neuron activity was suppressed during the kick there were no obvious IPSPs in the flexor at, or following, the moment of triggering. This provides further support for the suggestion that the M-neurons may be the only spiking interneurons providing direct inhibitory input to the flexor motoneurons at the time of triggering.

A Parallel Pathway for Terminating Flexor Activity

The lack of necessity of the M-neurons confirms that other neurons must be involved in repolarizing the flexors when a kick or jump is triggered. To date, only one other pair of metathoracic interneurons likely to play a role in the triggering process has been identified. These cells, one of which is shown diagrammatically in Fig. 3.13A, have been named 707 in accordance with the scheme proposed by Robertson and Pearson (1983). The pattern of activity of these neurons during bilateral kicks was very similar to the pattern seen in the M-neurons in that they were hyperpolarized throughout the co-contraction phase before discharging rapidly and strongly (Fig. 3.13B). In addition, a simultaneous recording (not shown) of an M-neuron and a 707 interneuron revealed the same grouping of spikes in their discharges during kicks, suggesting that these cells have a common source of excitatory input. It was no surprise, then, to find that in most cases the start of 707's burst coincided with the onset of repolarization in the flexor motoneurons (Fig. 3.14). However, as was also seen for the M-neurons, there were occasions on which 707 began to discharge after repolarization had commenced in the flexor. Here again, though, the timing of this repolarization matched the rising phase of the interneuron's depolarization. This indicates that still other neurons must contribute to the termination of flexor motoneuron activity.

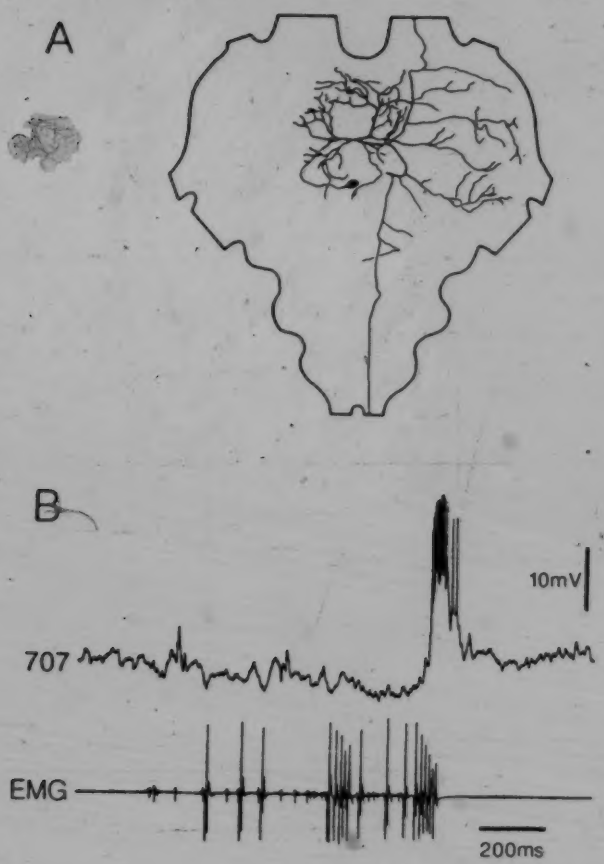

Fig. 3.13. Another pair of interneurons involved in triggering kicks. Diagram of the structure of the metathoracic interneuron 707 (A) and its pattern of activity during a bilateral kick (B). Top trace in B - intracellular recording from 707; bottom trace - EMG from the leg on the side of 707's numerous output branches (i.e. contralateral to its soma). Note that the activity of this cell during a kick was very similar to the pattern seen in the M-neuron (e.g. see Fig. 3.4).

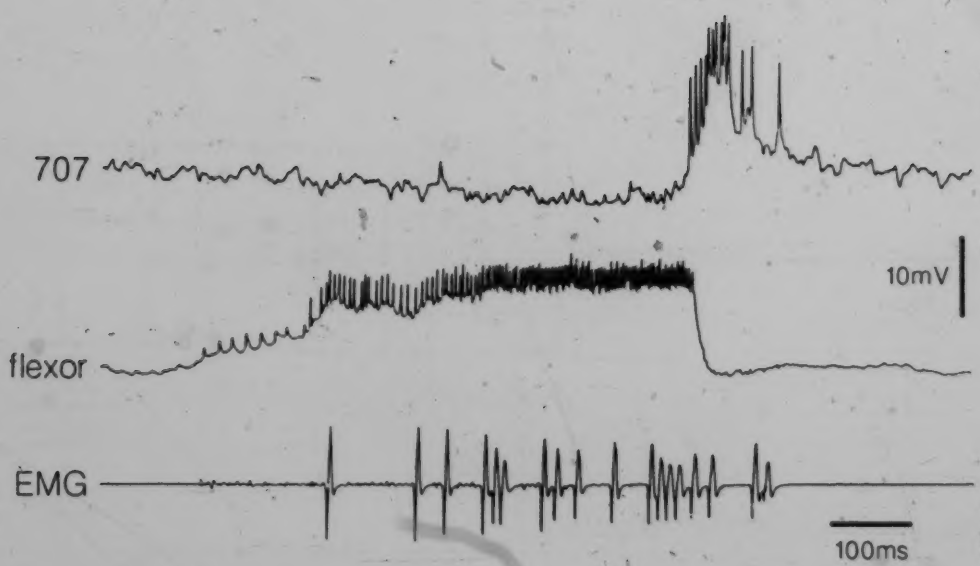

Fig. 3.14. The discharge in 707 occurs at the correct phase for it to contribute to the termination of flexor activity. Top trace - intracellular recording from a 707 interneuron; middle trace - a flexor motoneuron on the same side as this 707's axonal branches; bottom trace - EMG recording from the leg innervated by the flexor motoneuron. Note that the first spike in 707 corresponded precisely with the onset of repolarization in the flexor. The small spikes at the beginning of the flexor trace belonged to a different flexor motoneuron recorded with the same electrode.

Confirmation of the suspected involvement of the 707 neurons in the triggering process came from pulse experiments similar to those conducted for the M-neurons. A strong depolarizing current pulse in 707 during co-contraction caused the shutdown of flexor activity on the side of the interneuron's axonal branches (i.e. contralateral to the soma) and triggered the premature extension of this leg (Fig. 3.15A). As was the case for M, this effect was only observed when 707 was induced to discharge at frequencies well above normal, and the current pulse was not able to repolarize the flexor motoneuron as rapidly as occurs during a natural kick. However, the shape of this repolarization was not like that produced by a pulse in M (cf. Figs. 3.8, 3.9) but instead more closely resembled that seen during kicks in which M-neuron activity had been prevented (see Fig. 3.12).

According to the morphological criteria of Pearson and Robertson (1987), 707 is almost certainly an inhibitory interneuron. Unlike the M-neurons, however, 707 does not inhibit flexor motoneurons directly (Fig. 3.15B). This was determined for five different motoneurons (representing fast, intermediate and slow flexor categories) in three animals. Thus, as would be consistent with the proposal that the triggering process may also involve a shutdown of excitatory drive to the flexor motoneurons, 707's ability to terminate the activity of these flexors is most likely due to its inhibition of the interneurons responsible for flexor excitation during the motor program.

D. DISCUSSION

Organization of the Jump Trigger System

In Chapter 2 it was suggested that a system of interneurons within the metathoracic ganglion, referred to for convenience as the trigger system, is responsible for producing the burst of activity in the M-neurons at the end of the co-contraction period which in turn inhibits the flexor motoneurons and triggers the kick. In the present study it has been clearly established that the M-neurons do contribute to the process of triggering bilateral kicks (and

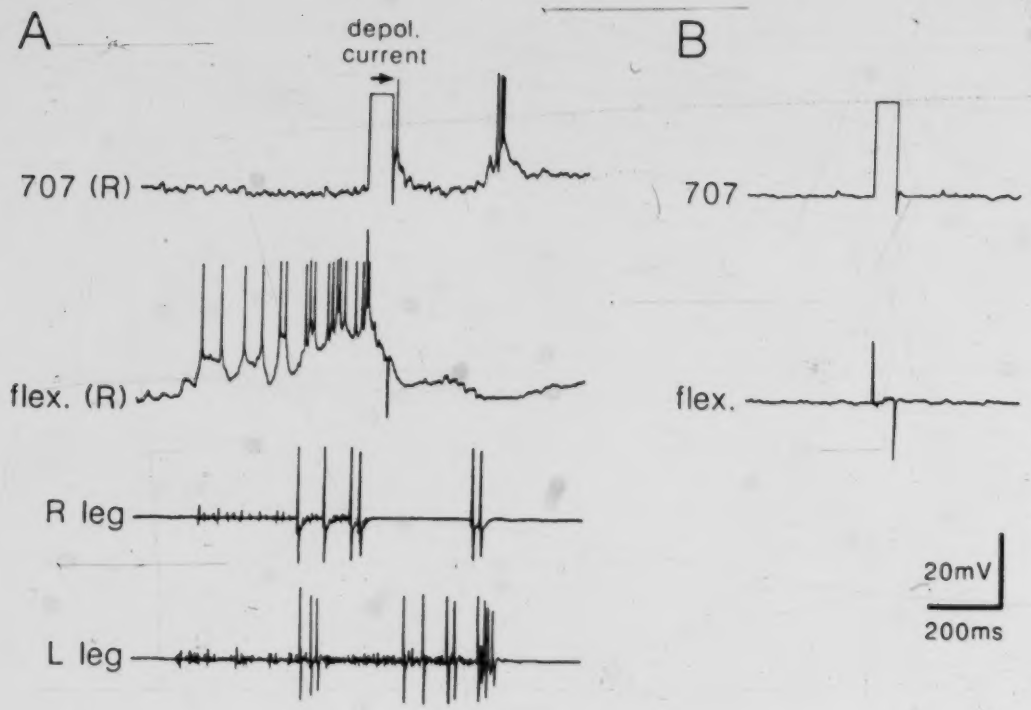

Fig. 3.15. Pulse-evoked activity in 707 can trigger leg extension during a bilateral kick. Top traces - intracellular recording from the 707 with its output branches on the right side of the metathoracic ganglion; second trace in A and bottom trace in B - right side flexor motoneuron; bottom traces in A - left and right leg EMGs. A A depolarizing current pulse applied to 707 during the co-contraction phase terminated spike activity in the flexor motoneuron and triggered extension of the right leg. The break in the series of extensor muscle spikes in the left leg EMG recording was a peculiarity of this particular animal because it also occurred during natural kicks. It was not caused by the pulse-evoked activity in 707. Note that the rate of repolarization of the flexor during the pulse in 707 was slower than occurs during a natural kick. B The same current pulse injected while the flexor motoneuron was at rest had no effect on the flexor's membrane potential (cf. Fig. 3.10B).

therefore jumps too, since the motor programs for these behaviors are similar) by way of this powerful inhibitory influence on the flexor motoneurons. However, the finding that the M-neurons were not necessary for kicking to occur demonstrates that additional neurons must play a role in terminating flexor activity during the final phase of the motor program. It is now proposed that the neuronal mechanism which underlies the shutdown of flexor activity may involve not only the direct inhibition of flexor motoneurons but also a concurrent cessation of excitatory input to the flexors. Here the evidence which supports this proposal will be reviewed.

It is quite obvious that the flexor motoneurons receive a strong, maintained excitation throughout the co-contraction period. Theoretically, if the M-neurons were able to produce a sufficiently powerful inhibition, it might be possible for them to overcome this ongoing excitatory drive and repolarize the flexor motoneurons to resting level. Yet it was found that pulse-evoked M discharges during co-contraction, even if of greater than normal frequency, were not able to cause as complete a repolarization of the flexor motoneurons as occurs during natural kicks (Figs. 3.8, 3.9). This strongly suggests that the inhibitory action of the M-neuron was weakened by the concurrent flexor excitation and that during the normal triggering process this excitatory input must also be shut off. However, a similar result would be expected if triggering depended on multiple inhibitory inputs to the flexor motoneurons, i.e. from neurons in addition to M. By depolarizing the M-neuron we would not activate these other inhibitory pathways and the resulting inhibition of flexors would be weaker than normal. Two observations, though, indicate that the M-neurons may be the sole source of direct flexor inhibition during trigger activity. The only discrete IPSPs occurring in the flexor motoneurons during kicks corresponded on a one to one basis with spikes in the M-neuron (Figs. 3.2, 3.3) and when M's activity was suppressed, IPSPs were no longer apparent in the flexor as it repolarized (Fig. 3.12). Unfortunately, these findings do not rule out the possibility that nonspiking interneurons also contribute to the inhibition of the flexors. However, one additional observation suggested that this may not be so and that the

termination of flexor activity which triggers kicks and jumps may depend only on the dual processes of M-neuron inhibition of flexors and the reduction of excitatory flexor drive. This was the greater than normal variability in the rates of flexor repolarization during kicks in which M-neuron activity was prevented. If the flexors received multiple inhibitory inputs, there should not be such variability after the M-neurons had been removed from the system. The fact that this variability existed was more consistent with the idea that the only remaining mechanism for terminating flexor activity was the shutting down of excitatory input to these motoneurons.

Support for this proposal came from the discovery of the 707 interneurons. At present it can only be assumed that these interneurons are inhibitory but there is a good basis for making this assumption. Pearson and Robertson (1987) have found for 12 different interneurons that when a cell possesses the same basic structure as 707 (specifically a ventromedial soma, laterally bowed primary neurite and contralateral axons) its synaptic function is always inhibitory. Since 707 does not inhibit flexor motoneurons directly (Fig. 3.15B), the only way to account for its ability to terminate flexor activity (Fig. 3.15A) is to suggest that it inhibits the interneurons responsible for flexor excitation. This would explain, then, why the nature of the flexor repolarization during kicks in which M-neuron activity had been abolished (Fig. 3.12) was similar to that produced by a pulse in 707 (Fig. 3.15A). This similarity would be expected if the same mechanism (i.e. a shutdown of excitation) were responsible for terminating flexor activity in both situations. Naturally, this idea would be strengthened if one or more interneurons were discovered which make excitatory connections with flexor motoneurons and whose activity is maintained throughout the co-contraction period, ending at the trigger point. The activity of such neurons will most likely be caused by afferent feedback from proprioceptors in the hind femora (Heitler and Burrows 1977b). Two neurons with this pattern of activity are known to exist in the metathoracic ganglion but their connections and synaptic actions have not yet been determined.

Because of the close similarity between the synaptic patterns and timing of discharges in 707 and M during bilateral kicks (compare Figs. 3.4 and 3.13), it is probable that the postulated central trigger system simultaneously controls the activity of both neurons. Consequently, the M-neuron inhibition of flexor motoneurons and the postulated 707 inhibition of the interneurons which excite flexors may form two parallel limbs of the output pathway by which the trigger system is able to terminate flexor activity and thereby trigger a kick or jump. However, the complete system must also include other cells because, on occasion, spike activity in M and 707 occurred after the flexor motoneurons had already begun to slowly repolarize (Fig. 3.3). Since this repolarization was coincident with the onset of the ramp depolarizations in both M and 707, it seems very likely that the activity of the cells which produce it is also under the control of the central trigger system. The reason why during slow ramp depolarizations these neurons are seen to exert their influence on flexors prior to the time M and 707 become active may either be because they are nonspiking or because they have lower spike thresholds than do M and 707. When the central trigger system produces a sudden depolarization of all the trigger neurons, as is more often the case, it is no longer possible to distinguish the different actions of the system's various output pathways because these are all activated simultaneously.

A possible candidate for one of these additional trigger neurons is the spiking local interneuron described in Chapter 2 (Fig. 2.9). It has the threshold characteristics and discharge pattern such a trigger neuron would be expected to possess. Moreover, this cell has an excitatory effect on the fast extensor motoneuron and so may also exert an inhibitory influence, either directly or indirectly, on the antagonist flexors. It is quite likely that such local neurons will be found to play a major role in the functioning of the trigger system.

The pattern of activity of the inhibitory flexor motoneurons, which serve to reduce tension in the flexor muscle, bears a striking resemblance to that seen in both 707 and M (see Heitler and Burrows 1977a, their Figs. 5 and 6). It is probable, therefore, that the activity of these motoneurons is also governed by the central trigger system and that they constitute yet

another of the system's output channels. The emerging picture is that the complete neuronal system for triggering kicks and jumps may be a complex one, organized such that the signal from the central trigger neurons is directed through multiple output pathways, each with a different action but all contributing to the common goal of achieving relaxation of the flexor muscle. From a functional standpoint, this would make a good deal of sense. A trigger system which operated by not only inhibiting the flexor motoneurons and their source of excitation, but also the flexor muscles, would ensure that tension in these muscles decreased as rapidly and as completely as possible. This is important because any lingering flexor tension would dampen the rate of extension of the tibiae, thereby decreasing the animal's jumping or kicking performance.

Role of the M-neurons in Triggering Kicks and Jumps

Due to the results of this, and the previous study (Chapter 2), the currently held view of the importance of the M-neurons must be altered. Originally these interneurons were thought to be critical, decision-making elements of the trigger circuitry because they receive convergent excitatory input from a variety of sensory sources known to be important for eliciting jumps (Pearson et al. 1980; Steeves and Pearson 1982). In this role, it was believed that the M-neurons decide when the animal will jump according to when the combination of sensory inputs brings their membrane potentials above threshold. The findings in the present series of investigations, however, indicate that the generation and timing of spike activity in the M-neurons is not directly influenced by the occurrence of external sensory stimuli (see Chapter 4), but rather depends on a higher level interneuronal trigger system (Chapter 2). Thus the M-neurons, instead of being key elements in the trigger circuitry, probably just constitute an output pathway between the trigger system and the flexor motoneurons. Even in this more restricted role it was quite feasible to think that the M-neurons might be indispensable components of the jump system, being the only neurons through which the signal to trigger a jump is channeled. However, the finding that locusts can kick when activity

in the M-neuron is prevented (Figs. 3.11, 3.12) has shown that even this is not true. It now seems probable that the M-neurons are just one output pathway in a complex system for terminating activity in the flexor motoneurons.

Two observations in the present study cast doubt on the importance of the role played by the M-neurons in this neuronal system for triggering kicks and jumps. The first was that the latency to hindleg extension was apparently unchanged by the absence of M-neuron activity (Figs. 3.11, 3.12). Secondly, although in the majority of kicks the discharge in the M-neurons began before the flexors had started to repolarize, at times this discharge followed the onset of repolarization and, occasionally, occurred after the flexor's membrane potential had already fallen below its threshold for spiking (Fig. 3.3). These results suggest that the most important event in the process by which kicks are triggered may not be the M-neuron inhibition of flexors but rather another mechanism for terminating flexor activity such as the shutdown of excitatory input to these motoneurons. Certainly when the M-neurons were active they were able to produce a more rapid repolarization of the flexor motoneurons (Fig. 3.12) but it is doubtful that this, in itself, has any functional significance. As far as triggering of the kick is concerned, the critical event is when the flexor motoneurons cease their spike activity because this is the moment at which tension in the flexor muscle will start to decay, subsequently enabling the leg to extend. If the membrane potentials of these motoneurons have already fallen below threshold, the rate at which tension in the muscle decreases will not be affected by how rapidly the motoneurons are further repolarized.

What, then, can the value of M's contribution be? Unfortunately this is only a matter for speculation. One possibility is that the M-neurons may help to ensure synchrony in the shutdown of flexors that might be difficult to achieve if this depended solely on the reduction of excitatory drive to the flexor motoneurons. If activity in all the flexors did not end at the same instant, any additional spikes would tend to oppose the decay in tension in the flexor muscle, thereby opposing the extension of the tibia. If this idea were true, however, there should be a prolongation of the latency to hindleg extension when M-neuron activity during a

kick is abolished. There are two reasons which might explain why such an effect was not seen. The first of these is related to the inadequacy of the data. Spike activity in the M-neurons was only prevented on two occasions: in one animal while monitoring extension of the hindleg with a photocell (Fig. 3.11) and in another while recording from a flexor motoneuron (Fig. 3.12). The reliability of the photocell results is questionable because it was only possible to estimate the latency rather than to measure it accurately. Also, the conclusions about the effects of M's hyperpolarization on the flexor motoneuron were based solely on a comparison of two normal kicks and four kicks in which M-neuron activity had been suppressed. Perhaps more extensive data would show that the M-neurons do exert an influence on the timing of flexor shutdown and leg extension. However, to obtain definite proof that M acts to synchronize flexor shutdown would require recording the activity of two or more flexor motoneurons innervating one leg, before and after the appropriate M-neuron had been removed from the system (e.g. by cell killing techniques). This would reveal whether or not, in the absence of M's activity, spiking in these motoneurons is terminated simultaneously.

A second reason why preventing the M-neuron's discharge may have had no noticeable effect on the timing of leg extension could be related to the behavioral situation. In these experiments the animal was allowed to kick freely, without anything to resist tibial extension. During a jump, however, the hind tibiae push against the ground during take-off so that the locust's own weight will act as a force opposing the legs' extension. Consequently, after the jump has been triggered there may be a considerable amount of proprioceptive input which might tend to prolong activity in the flexor motoneurons. In this situation the M-neurons may be of vital importance in preventing such a prolonged flexor discharge from occurring. If this were true, it could no longer be claimed that the preparation in which a locust performs bilateral kicks accurately reflects the events which occur during a jump.

Comparison With Other Systems

It may not be entirely appropriate to make direct comparisons between the jump trigger system in the locust and the various systems which initiate escape responses in other animals (e.g. Wine and Krasne 1982; Eaton and Hackett 1984; Nolen and Hoy 1984). All of these systems function to elicit motor responses but the major difference in the case of the locust jump is that the signal which triggers the movement terminates the entire motor program rather than initiating it. Thus, activating the jump trigger system could only be expected to trigger the behavior if there had been a prior excitation of the flexor and extensor motoneurons to produce co-contraction in the hindlegs. Nevertheless, interesting parallels can be drawn between our findings for the M-neurons and what is known about identified neurons in other escape systems. These analogies apply, in particular, to the reticulospinal Mauthner cells which are involved in the escape response of teleost fish. The Mauthner neurons were once considered to be solely responsible for eliciting the fast-start escape movement in response to a sudden visual, auditory or vibratory stimulus, but it is now known that these cells are not necessary for all such fast-starts (Eaton and Bombardieri 1978; Kimmel et al. 1980; Eaton et al. 1982). Furthermore, the Mauthner neurons cannot, by themselves, evoke the normal strength and variety of escape responses that a fish displays when subjected to a particular sensory stimulus (Eaton 1986). Current thinking is that escape behavior in fish is activated by a system of these reticulospinal trigger neurons. Thus, as for the triggering of the locust jump, parallel pathways exist for initiating the movement. This sort of parallel organization is not restricted to systems eliciting escape responses but is also evident in the initiation of rhythmic behaviors such as swimming in the leech (Nusbaum and Kristan 1986) and feeding in pond snails (McCrohan 1984). In the latter example, though, the two pathways are probably not activated together.

In all of these systems which initiate movement, the way in which sensory input functions to elicit the various responses is well understood. Sensory afferents of one or more modalities either directly or indirectly excite the neurons which trigger the escape or rhythmic

behaviors. Thus, for instance, the lateral giant interneurons which generate the crayfish tailflip receive both direct and disynaptic input from abdominal mechanoreceptors (Wine and Krasne 1982). Similarly, the gating neurons 204 and 61 which elicit swimming in the leech also receive disynaptic mechanosensory input (Brodfuehrer and Friesen 1986). Numerous other examples also exist. In the locust jump system, however, the way in which sensory input leads to the generation of the trigger signal is only just beginning to be understood. The neurons directly involved in terminating flexor activity (e.g. the M-neurons) do receive afferent input, but it does not appear to be important for generating their trigger discharges. Rather, the sensory input probably activates these neurons indirectly via the postulated central trigger system. This key issue of how sensory information contributes to the triggering process will be addressed in the following two chapters.

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IV. EVIDENCE THAT EXTEROCEPTIVE INPUT DOES NOT TRIGGER KICKS AND JUMPS

A. INTRODUCTION

The ballistic movements associated with kicks and jumps in the locust are produced by a motor program consisting of three distinct phases (Godden 1975; Heitler and Burrows 1977a). During the first phase the hind tibiae are flexed about the femora. There is then a prolonged period of co-contraction of the flexor and extensor tibiae muscles, during which time the energy that powers the ensuing movement is stored in elastic cuticular elements of the femoral-tibial joint. Finally, during the trigger phase, the flexor muscle suddenly relaxes, allowing the stored energy to be released as the hindlegs forcefully extend. To understand how this triggering is brought about it is necessary to determine what is ultimately responsible for generating the signal to terminate flexor activity.

It was originally suggested by Pearson et al. (1980) that jumps and kicks may be triggered by extrinsic sensory stimuli activating a pair of metathoracic interneurons, the M-neurons, whose excitability is regulated by proprioceptive input. These M-neurons were known to make powerful inhibitory connections to hindleg flexor motoneurons. According to the Pearson et al. (1980) hypothesis, proprioceptive feedback during the co-contraction phase gradually depolarizes the M-neurons, bringing them closer to threshold. Additional excitatory input from a sudden exteroceptive stimulus would then drive the membrane potential of the M-neurons above threshold and the resulting burst of spikes would inhibit the flexor motoneurons and trigger the kick or jump. This idea was based primarily on knowledge of the excitatory inputs to the M-neurons, and on the fact that these interneurons inhibit flexors. Both members of the pair receive strong monosynaptic input from the descending contralateral movement detector (DCMD) neurons and are depolarized by auditory and tactile stimuli. They also receive excitation from hindleg proprioceptors. Because of the high threshold of the M-neurons, input from one or more of the exteroceptive modalities rarely

initiates action potentials, but when a combination of exteroceptive and proprioceptive inputs are presented there is a high probability of spikes being evoked in the M-neuron (Steeves and Pearson 1982). Also, recordings made in kicking locusts revealed that the M-neurons discharge at the end of co-contraction, and this is preceded by a slow depolarization of their membrane potentials (Steeves and Pearson 1982). Furthermore, the onset of this M-neuron activity was observed to closely follow the presentation of visual or auditory stimuli.

Recent studies, however, have yielded results which are not consistent with aspects of this hypothesis. In Chapter 2 it was shown that the M-neurons are hyperpolarized during co-contraction, rather than being depolarized as Steeves and Pearson (1982) had observed. As yet, no satisfactory explanation for the discrepancy between these two observations has been offered. Also, Heitler (1983) found that during the co-contraction phase of defensive kicks in restrained animals, the activity of the DCMD neurons is suppressed, indicating that DCMD may not be capable of eliciting trigger activity. However, Heitler suggested that a natural stimulus may be sufficiently powerful to overcome DCMD's suppression, so that these visual interneurons might still be able to trigger the behavior.

In the light of these recent results, the notion that defensive kicks and escape jumps can be triggered by a summation of proprioceptive and exteroceptive inputs seemed questionable. It was, therefore, the main purpose of the experiments described here to directly test this hypothesis by determining whether the M-neurons can be activated by sudden visual or auditory stimuli presented during the co-contraction phase. The results show that these stimuli are not able to trigger kicks and in fact evoke depolarizations of smaller amplitude in the M-neuron than they do in the quiescent animal. This is not because of suppression of activity in the sensory pathways, but probably because the depolarizations are shunted by the increased conductance during the M-neuron's hyperpolarization. Thus, it is concluded that exteroceptive information is probably not involved in the process by which defensive kicks and escape jumps are triggered. Its importance, instead, undoubtedly lies in its ability to initiate the entire motor program for these behaviors. Also, a solution is provided to the apparent

contradiction concerning the sign of M's input during co-contraction, thereby explaining why the idea of direct proprioceptive gating of the M-neuron's activity had initially received support. In the following chapter the contribution of proprioceptive input to the generation of the motor program and its trigger signal is assessed.

B. MATERIALS AND METHODS

Preparation of Animals

All experiments were performed at room temperature (22-24°C) on adult male *Locusta migratoria* from a long-established colony at the University of Alberta. The animals were prepared for intracellular and EMG recording as described in Chapter 2. In some experiments a monopolar hook electrode (75 µm silver wire) was placed on the right pro-mesothoracic connective to monitor the activity of the left DCMD interneuron. Kicks were elicited from the dissected animals by stroking or pinching their abdomens with a pair of forceps.

Intracellular Recording and Dye-filling

Glass microelectrodes (resistance 30-80 megohms), containing either 1M potassium acetate or a 5% aqueous solution of Lucifer Yellow, were used to record intracellularly from the M-neurons and the auditory G-neurons in the mesothoracic ganglion (Rehbein 1976). When necessary, neurons were filled with dye at the end of the experiment by passing a constant hyperpolarizing current of 5 nA for up to 5 minutes. The ganglia were then fixed in 4% paraformaldehyde, dehydrated in alcohol and cleared in methyl salicylate. Wholemounts of the ganglia were viewed under an epifluorescence microscope.

All recordings were stored on magnetic tape and later displayed on a Gould ES1000 electrostatic chart recorder.

Presentation of Sensory Stimuli

The presentation of sudden visual and auditory stimuli during co-contraction was timed using a digital device which counted extensor muscle spikes in the hindleg EMG recording. After a specified number of spikes had occurred within a chosen time window, the device produced an output pulse which was used to trigger a stimulator. The output of the stimulator, in turn, was used to drive devices for producing movements in the visual field or pulses of sound (see below). In this way, stimuli could be presented at different stages of the co-contraction phase.

The visual stimulus used to evoke DCMD activity in these experiments was the sudden movement of a cardboard disc (1.5 cm in diameter) placed level with, and 10 cm away from, the locust's left eye. The disc was mounted on the end of a thin rod, 40 cm in length. The other end of the rod was bent at a 90° angle and glued to the center of a speaker diaphragm. Through a lever arrangement, movements of the speaker coil (driven by an audio amplifier) were translated into vertical movements of the disc over an arc of approximately 6 cm. Following the initial large deflection, smaller movements of the disc continued for a brief period until oscillations in the rod subsided. Consequently, three or more bursts of spike activity, of diminishing intensity, were often evoked in the left DCMD neuron.

It should be noted that whereas activity of the DCMDs is suppressed during kicks in intact and minimally dissected animals, these neurons can be activated during co-contraction in the dorsal preparation (Heitler 1983).

Auditory stimulation was achieved using 60 dB, 100 ms white noise pulses, delivered through a high frequency speaker (Speaker Lab., Model DT101) positioned approximately 35 cm from the animal's right side.

C. RESULTS

Support for the idea that the kick or jump motor program can be triggered by a summation of exteroceptive and proprioceptive inputs had come from the observation of Steeves and Pearson (1982) that the M-neurons are gradually depolarized during co-contraction. Recordings presented in Chapter 2, though, showed that the M-neurons are hyperpolarized during co-contraction. The initial experiments here were aimed at resolving this apparent conflict by re-examining the synaptic potentials in the M-neuron.

Intracellular recordings from the axonal branch of the M-neuron (in the lateral neuropile region) usually reveal unitary synaptic potentials which are attenuated versions of those seen in the neuron's medial processes. This indicates that the M-neuron receives inputs on its medial processes and the postsynaptic potentials generated there simply conduct electrotonically out into the lateral branch (Pearson et al. 1980). However, it is now apparent that the assumption that recordings of synaptic activity from the lateral branch always reflect events occurring elsewhere in the neuron is incorrect. During the co-contraction phase that precedes hindleg kicks, simultaneous recordings from the medial and lateral processes revealed that although the medial branches were hyperpolarized, the lateral branch often exhibited a distinct depolarization prior to the neuron's burst of spikes. In the example shown in Fig. 4.1 the slow time course of the depolarization resembled that seen in the recordings of Steeves and Pearson (1982, their Figs. 11 and 12). More typically, the depolarization was not ramp-like, but instead exhibited a steady or gradually waning level throughout the co-contraction phase (Fig. 4.2A). There was no consistent correlation between the particular pattern of depolarization and the shape of the hyperpolarization in the M-neuron's medial processes. In some recordings, no maintained depolarization in the lateral branch was evident at all (Fig. 4.2B). It was not possible to determine whether this variability in the occurrence and nature of the depolarizations in these recordings was due to differences between animals or to differences between the exact site in which the M-neuron was penetrated. All recordings were made in the general region in which the lateral branch synapses with flexor motoneurons, but

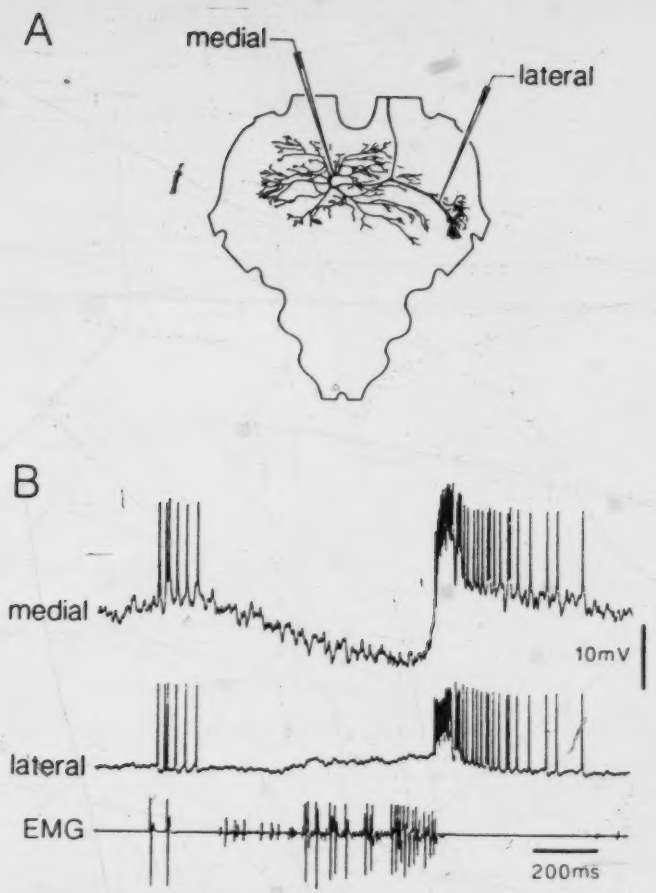

Fig. 4.1. Medial and lateral processes of the M-neuron can receive different synaptic inputs during bilateral kicks. **A** Diagram of the intracellular recording setup. The M-neuron was penetrated with two electrodes, one placed in the neuron's medial processes and the other in the lateral axonal branch in the region of the flexor motoneurons. **B** Dual intracellular recordings from the same M-neuron during a bilateral kick. Only the EMG from the leg on the side of M's lateral branch is shown here (bottom trace). During the co-contraction phase the M-neuron's medial processes (top trace) were hyperpolarized while the lateral branch (middle trace) was gradually depolarized. Note that spike activity was identical at the two different sites.

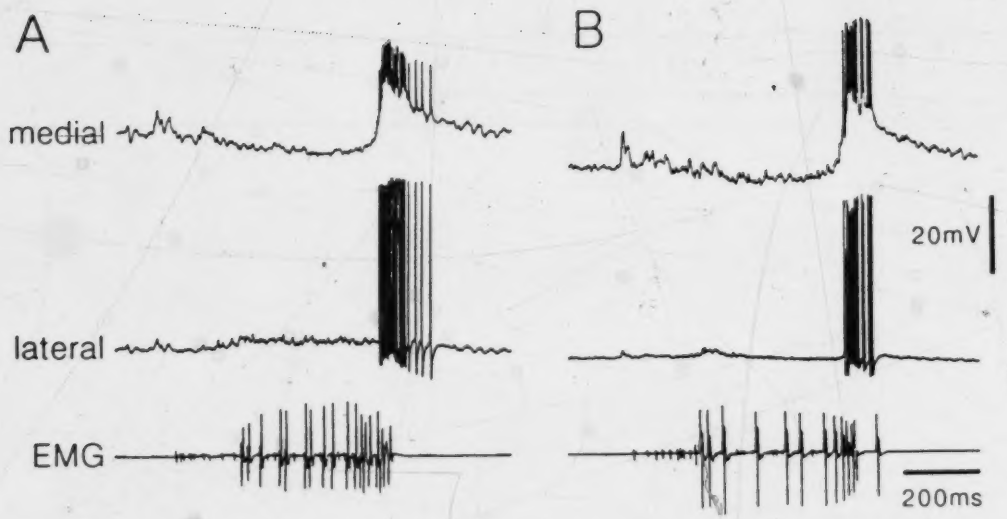

Fig. 4.2. Variations in the pattern of synaptic activity in the M-neuron's lateral branch during kicks. Examples of simultaneous intracellular recordings from the medial (top traces) and lateral (middle traces) processes of the M-neuron in two different animals. In each case, only the EMG from the leg on the side of the M-neuron's lateral branch is shown (bottom traces). During the co-contraction period the medial processes were always hyperpolarized, but most lateral branch recordings revealed a slight maintained depolarization, as shown in A. In other recordings from the axonal branch there was no obvious depolarization leading to M's discharge (B). Note that, prior to the onset of co-contraction, the two different regions of the neuron exhibit common synaptic potentials, although these are attenuated in the lateral branch.

within this region the M-neuron possesses numerous processes of various diameter. It is possible that recordings from processes of different size may not show identical features.

When a depolarization was apparent in the M-neuron's axonal branch, its onset did not usually coincide with the beginning of the hyperpolarization in the medial processes (see Fig. 4.1B). The latter typically commenced at the start of the initial flexion phase of the motor program (see also Chapter 2), whereas the depolarization often began at, or just prior to, the onset of co-contraction. However, during kicks in which the initial flexion period was very brief, i.e. with co-contraction commencing almost immediately, the depolarization in the lateral branch sometimes preceded the onset of both flexor and extensor EMG activity.

It is important to note that even though M's axonal branch may be depolarized during co-contraction, this does not contribute to the generation of action potentials in the cell. A continuous train of spikes cannot be evoked in the M-neuron by injecting the lateral branch with depolarizing current (Pearson et al. 1980). Thus, it is the synaptic inputs to the M-neuron's medial processes that govern its spike activity, and there is no doubt that the neuron is inhibited during the co-contraction phase. This was clearly demonstrated by injecting a constant depolarizing current of 6 nA into one of the M-neuron's larger medial processes to evoke tonic spike activity and observing that this activity was completely suppressed during co-contraction. Thus, it can be concluded with confidence that proprioceptive feedback does not progressively increase the excitability of the M-neurons prior to the trigger phase of the kick. This aspect of the Pearson et al. hypothesis must therefore be abandoned. However, it is possible that the remaining features of the original model, i.e. that exteroceptive inputs during co-contraction can trigger kicks, may still hold true. This possibility was tested by recording from the M-neurons (penetrated in their medial processes) while sudden visual and auditory stimuli were delivered during the co-contraction phase.

Figure 4.3 shows the effect that sudden movements in the left visual field exerted on the M-neuron while the locust was quiescent and while it was performing a bilateral kick. In both situations, the visual stimulus elicited a strong burst of activity in DCMD (recorded here

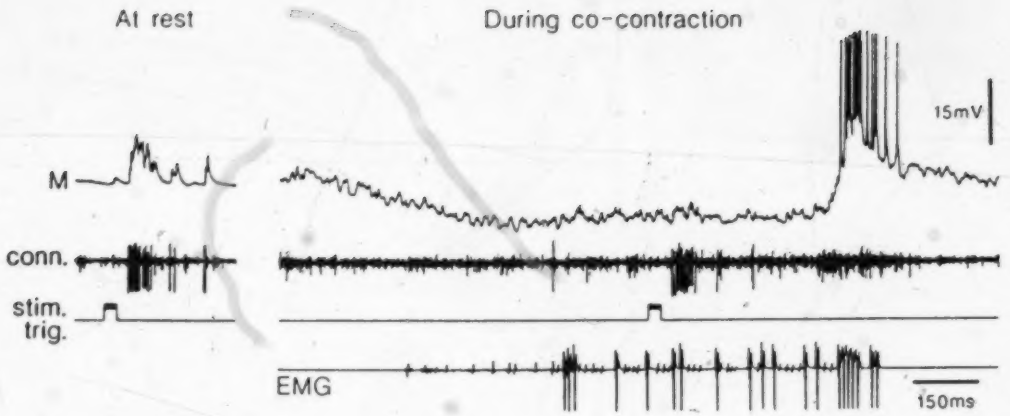

Fig. 4.3. The effect of visual stimulation on the M-neuron in the quiescent animal and during co-contraction. Top traces - intracellular recordings from the medial processes of an M-neuron; second traces - extracellular hook electrode recordings from the right pro-mesothoracic connective, monitoring activity of the left DCMD neuron; third traces - pulses used to trigger the visual stimulus; fourth trace (during the kick only) - EMG from the leg on the side of this M-neuron's axonal branch. The visual stimulus evoked an intense burst of DCMD activity (large spikes in connective recording) while the animal was at rest and while it was performing a bilateral kick. Note that the stimulus-evoked depolarization in the M-neuron was much weaker during the co-contraction phase than it was at rest. Also, during co-contraction it is difficult to distinguish individual EPSPs in the M-neuron occurring in response to single DCMD spikes.

from the right pro-mesothoracic connective), and yet when presented during co-contraction, it failed to trigger the kick. Following the stimulus, co-contraction continued for several hundred milliseconds before the trigger discharge occurred in the M-neuron. Furthermore, visual stimulation did not depolarize the M-neuron as strongly during co-contraction as it did at rest. The depolarization produced by the major burst of DCMD spikes was less than half the amplitude of the corresponding depolarization in the quiescent animal, and the EPSPs to subsequent DCMD spikes during co-contraction could hardly be discerned at all. The M-neuron displayed these smaller amplitude depolarizations regardless of when the stimulus was delivered during co-contraction. Bursts of DCMD spikes occurring late in the co-contraction phase were just as ineffective at producing trigger activity as those occurring earlier. In all cases, the size of the DCMD-evoked depolarization was insignificant in comparison with the magnitude of the depolarization M experienced during its trigger discharge.

Similar experiments were performed using sudden auditory stimuli presented during co-contraction. The M-neuron receives monosynaptic excitation from the auditory G-neurons in the mesothoracic ganglion, and probably also direct input from some first order auditory afferents which run in the metathoracic nerves 6 (Pearson et al. 1980). Brief pulses of white noise, which elicited large subthreshold depolarizations in the M-neuron in the quiescent animal, were not able to produce trigger activity when delivered during the co-contraction phase (Fig. 4.4). Moreover, just as had been noted for visual stimuli, the depolarization in M produced by auditory stimulation was greatly diminished during co-contraction. The sound pulses normally evoked depolarizations consisting of two distinct components - a strong phasic excitation, followed by a weaker tonic depolarization. During co-contraction, however, only the first component was visible and its amplitude was reduced by approximately 50%. Again, this result was not dependent on the timing of the sensory stimulus during co-contraction. In the example shown in Fig. 4.4, the sound pulse came towards the end of the co-contraction phase, when, according to the basic notion of the Pearson et al. proposal,

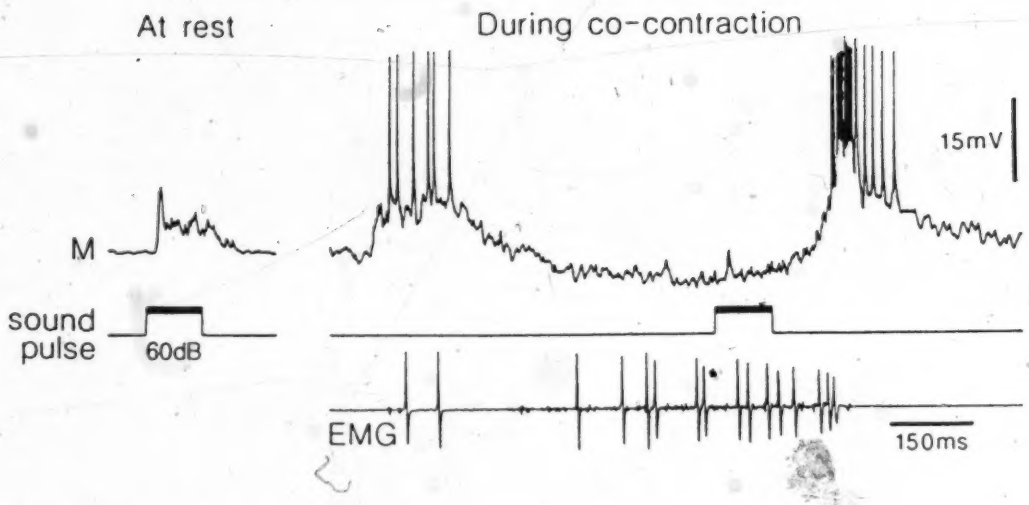

Fig. 4.4. The effect of auditory stimulation on the M-neuron in the quiescent animal and during co-contraction. Top traces - intracellular recordings from the medial processes of an M-neuron; second traces - auditory stimuli (60 dB, 100 ms white noise pulses); third trace (during the kick only) - EMG from the leg on the side of this M-neuron's lateral branch. Note that the depolarization produced by the sound pulse during the co-contraction phase was greatly reduced compared to its amplitude at rest. Only the initial phasic component of the depolarization is evident and even this is much smaller than in the quiescent animal.

the likelihood of it producing trigger activity would have been expected to be greater. Also, as was observed for DCMD stimulation, the depolarization in M elicited by the sound pulse was far smaller than that which produced the neuron's powerful trigger discharge at the end of the motor program.

The weak excitatory effect of these auditory stimuli during co-contraction could not have been due to any suppression of the activity of tympanal receptors while the behavior was being performed because the G-neuron's response to white noise pulses was the same in the quiescent and kicking animal (Fig. 4.5). The number of spikes elicited in the G-neuron by sound pulses at rest (mean=13.8, S.D.=1.99, n=28) did not differ from the number elicited during co-contraction (mean=14.4, S.D.=2.35, n=12; Student's t-test, $p>0.05$). Clearly then, auditory input was normal during the kick motor program and so, barring any presynaptic inhibitory effects, the G-neuron must have been providing its normal excitatory input to the M-neurons during co-contraction.

Since there was no apparent change in the level of activity in the visual and auditory pathways during the co-contraction phase, why did exteroceptive stimuli produce smaller depolarizations in the M-neuron at this time? The most probable explanation is that the excitatory inputs to M were shunted by the increased conductance caused by the neuron's inhibition during co-contraction. If such a postsynaptic mechanism were responsible, one might expect to see a gradual decrease in the amplitude of stimulus-evoked depolarizations in M as the neuron became hyperpolarized at the onset of the motor program. Unfortunately, because of the difficulty in timing the presentation of sensory stimuli during the initial flexion period, it has not been possible to determine whether this does occur. However, in an attempt to demonstrate the same phenomenon, conductance tests were performed on the M-neuron. Brief pulses of depolarizing current were applied to M throughout the motor program, but no decrease in the amplitude of the resulting voltage deflections was apparent during the neuron's hyperpolarization. Despite this inability to detect a change in conductance in M's medial processes during co-contraction, it is still likely, as is explained in the Discussion, that such a

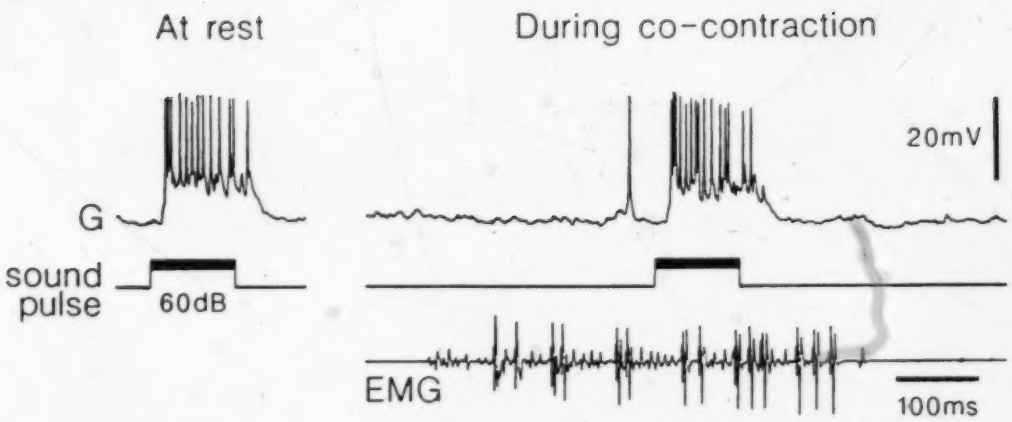

Fig. 4.5. Response of the G-neuron to auditory stimulation in the quiescent animal and during co-contraction. Top traces - intracellular recordings from the G-neuron in the mesothoracic ganglion; second traces - auditory stimuli (60 dB, 100 ms white noise pulses); third trace (during the kick only) - EMG from one of the hindlegs. The G-neuron responded as intensely to the sound pulse during co-contraction as it did at rest, indicating that auditory input was not suppressed during the kick motor program.

change was the major factor contributing to the reduction in amplitude of the depolarizations in the M-neuron. The possibility that presynaptic inhibition may also reduce the effectiveness of exteroceptive inputs to the M-neurons during co-contraction has not been excluded.

D. DISCUSSION

The main finding of the present study is that exteroceptive stimuli presented during the co-contraction phase are not capable of triggering kicks in the dissected preparation. Consistent with this is the observation that the timing of the M-neurons' trigger discharges did not coincide with the presentation of sensory stimuli. Moreover, the exteroceptive inputs to M did not excite the interneuron as strongly during co-contraction as they did normally. These findings do not support the basic prediction of the Pearson et al. (1980) model that a summation of exteroceptive and proprioceptive inputs is required to produce trigger activity in the M-neurons. They also contrast with the results of an earlier study which indicated that the triggering of kicks was contingent on the occurrence of extrinsic sensory stimuli (Steeves and Pearson 1982). In this discussion the probable reason for a major discrepancy in the recent experimental data will be considered, before reviewing the evidence which supports the idea that exteroceptive inputs are not involved in the triggering process.

Hyperpolarization or Depolarization? A Conflict Resolved

The proposal that the excitability of the M-neurons is gradually increased during co-contraction due to feedback from hindleg proprioceptors was based on two main observations. Firstly, the M-neurons were known to receive excitatory input from the chordotonal organ and lump receptor in the hind femora, two proprioceptors which had been implicated in the control of jump triggering (Bässler 1968; Heitler and Burrows 1977b; Pearson et al. 1980). Secondly, the recordings of Steeves and Pearson (1982) showed that during defensive kicks the M-neurons are slowly depolarized prior to their trigger bursts. Together, these observations provided what had seemed like convincing evidence in support of

the proprioceptive gating hypothesis. It was quite puzzling, therefore, when subsequently it was found that M is not depolarized, but in fact hyperpolarized, during co-contraction (Chapter 2). No explanation has been offered for the obvious discrepancy between this and the earlier findings. The results of the present investigation, however, now suggest a plausible solution to this apparent conflict. Simultaneous recordings from the medial and axonal processes of the same M-neuron revealed that the two regions can receive synaptic input of opposite sign during co-contraction. While the medial processes of the neuron were hyperpolarized, the axonal branch sometimes displayed a prolonged depolarization (Figs. 4.1, 4.2). It is therefore probable that the recordings of Steeves and Pearson (1982) were from a penetration of the M-neuron made somewhere in the lateral branch. This is also suggested by the nature of the action potential bursts in their recordings. These are not superimposed upon pulse-like depolarizations, and each spike in the burst exhibits a distinct after-hyperpolarization (see their Figs. 11 and 12). Both of these features are typical of recordings from a neuron's axonal region. With this conflict in the experimental data now resolved, the proposal that proprioceptive feedback brings the membrane potential of the M-neurons closer to threshold can be confidently rejected.

The depolarization of the lateral branch appears to have a central origin, rather than being dependent on reflex input from the hindlegs, because its onset sometimes preceded the start of all EMG activity during kicks. Its functional significance is unknown, but one suggestion is that the depolarization may be due to presynaptic inhibition of the M-neuron's output terminals. This would seem to be appropriate. If the M-neuron's major site of input (its medial branches) are inhibited during co-contraction, presumably to prevent M from interrupting the ongoing motor program by becoming active prematurely, it would make sense to also presynaptically inhibit its output sites. This would serve to decrease the amplitude of the IPSP produced in flexor motoneurons by a possible premature M-neuron spike and thus further reduce the risk of early triggering. The observed variability in the occurrence of the depolarizations in lateral branch recordings may be related to the exact site of recording, since

presumably the amplitude of the depolarization would depend on the proximity of the electrode penetration to the neuron's output terminals. It is possible, for instance, that a recording from the main lateral branch might show no sign of presynaptic inhibitory input (perhaps as in Fig. 4.2B), whereas recordings from finer processes in this region may resemble those shown in Figs. 4.1B, 4.2A. To obtain proof that the M-neuron is presynaptically inhibited prior to the trigger phase would require demonstrating that the axonal depolarization is increased in magnitude by the injection of chloride ions, or that it causes a reduction in the amplitude of action potentials evoked in M during co-contraction.

Exteroceptive Inputs Do Not Trigger Kicks

There is now evidence that there are other neurons, in addition to M, involved in producing relaxation of the flexor tibiae muscle during the trigger phase, and that the activity of all of these neurons is controlled by some central trigger system (Chapters 2,3). Despite this, the essence of the original hypothesis that defensive kicks and escape jumps may be triggered by a combination of proprioceptive and exteroceptive inputs could still be valid if trigger activity were produced by a convergence of these inputs at the level of this central system. This would then be reflected in the activity of neurons such as M. Another possibility is that even though the M-neuron and other neurons directly involved in terminating flexor activity are inhibited during co-contraction (see Chapter 3) the strength of the excitatory inputs they receive from extrinsic sensory sources may be sufficient to overcome their inhibition, thereby causing them to discharge and eliciting trigger activity. The results of the present study, however, suggest that neither of these proposals is correct. During the co-contraction phase strong exteroceptive input (from sudden visual or auditory stimuli) was not able to trigger kicks, either by activating the hypothetical central system of interneurons or by direct excitation of the M-neurons (Figs. 4.3, 4.4).

Heitler (1983) has previously shown that under experimental conditions the DCMD neurons are not involved in triggering defensive kicks because their activity is suppressed

during this behavior. He was not able to determine whether DCMD suppression also occurs during jumping, but argued that this is likely. However, it was suggested that a natural stimulus might be more powerful than an experimental one, and so may still be able to evoke spikes in DCMD and trigger a kick or jump. The present results indicate that this is improbable because even strong bursts of DCMD activity were not capable of triggering bilateral kicks in our preparation (Fig. 4.3). Similarly, auditory stimuli failed to produce trigger activity in these dissected locusts (Fig. 4.4). These observations differ from those of Steeves and Pearson (1982, their Fig. 11) who reported that the timing of the M-neuron's discharge closely follows the occurrence of visual and auditory stimuli. The belief now is that this earlier finding may have been due to the reaction time between the experimenter perceiving the onset of co-contraction and manually delivering the sensory stimulus.

Despite these findings, the possibility cannot be ruled out that extrinsic sensory stimuli may be able to elicit trigger activity in intact locusts because of the higher levels of central arousal these animals possess. Any such triggering would be unlikely to result from a direct activation of the M-neurons (and other neurons which terminate flexor activity) because the excitatory effect of the exteroceptive inputs to these neurons is weakened during co-contraction. Triggering might result, though, from the extrinsic stimuli exciting the proposed central trigger system. Nevertheless, it is clear in the present experiments that exteroceptive inputs played no role in generating the powerful discharges in the M-neurons during the trigger phase. Therefore, it seems feasible to suggest that whatever does produce this trigger activity in the dissected preparation may be mostly, if not entirely, responsible for triggering kicks and jumps in intact animals as well.

The most probable reason that the M-neuron exhibited diminished responsiveness to sensory stimuli delivered during co-contraction is that its medial processes were strongly hyperpolarized at this time. Excitatory inputs to these processes would, therefore, have been considerably shunted. The failure to demonstrate a corresponding increase in conductance in the M-neuron was probably because the shunting phenomenon is localized to the fine

dendritic branches where M receives its synaptic inputs. The intracellular recording and current injection sites were in the largest of the medial processes, far removed from these input regions. Consequently, EPSPs would be shunted prior to reaching the site of the electrode penetration. The occurrence of such "dendritic remote inhibition" has been demonstrated in the Mauthner cell of the goldfish (Diamond 1968), and Currie and Carlsen (1987) suggest that this same phenomenon may contribute to the arousal-associated decrease in vibration sensitivity of the Mauthner neurons in larval lampreys. This decreased sensitivity is thought to prevent startle responses from being elicited at inappropriate times by the lamprey's own movements. The hyperpolarization of the M-neurons (and other trigger neurons) during co-contraction may serve a similar function by helping to ensure that they are not activated before the entire motor program has been played out, thereby preventing an ineffective kick or jump from occurring. Suppression of activity in sensory pathways (as was described for DCMD by Heitler 1983) and presynaptic inhibition of sensory inputs to the trigger neurons (as also described for DCMD, Pearson and Goodman 1981) would act to further reduce the probability of exteroceptive input prematurely triggering a jump.

If each M-neuron receives direct sensory input from a variety of sources and yet this plays no role in generating its trigger discharge during kicks and jumps, what purpose does this input serve? One idea is that the M-neurons may be involved in controlling the tibiae during other behaviors in which these inputs are important. However, there is as yet no evidence to implicate the M-neurons in any additional behavior. Even during walking, which involves repeated flexions and extensions of the hindleg tibiae, the activity of the M-neurons is weak and not correlated with the motor pattern (J.-M. Ramirez, personal communication).

In hindsight, it does not seem particularly surprising that exteroceptive sensory inputs may not be involved in the process of triggering kicks or jumps once co-contraction is already underway. In the experimental situation, locusts sometimes kick spontaneously when no threatening stimulus is apparent. Locusts also jump purely as a means of locomotion. In addition, as was already mentioned (see also Heitler 1983), for a locust to kick or jump

effectively it is important that co-contraction not be interrupted before the extensor muscle has developed maximum tension. If extrinsic sensory stimuli were capable of evoking trigger activity, such premature triggering would sometimes occur. This does not mean, however, that exteroceptive information is of no importance for these kicking and jumping behaviors. In fact, it makes a critical contribution to the initiation of the entire motor program for defensive kicks and escape jumps. In intact, aroused animals, visual, auditory and tactile stimuli can elicit the "cocking response", which is often a prelude to co-contraction (Pearson 1981; Pearson and Robertson 1981). In dissected locusts, with lower levels of arousal, this particular response is abolished, but a motor program commencing instead with a period of "initial flexion" can be elicited by tactile stimulation. However, for spontaneous kicks and locomotory jumps to occur, it is obvious that there must be other means by which the motor program can be initiated. Descending commands from the brain are probably important in this respect.

Pearson (1981, 1983) suggested that the sensory mechanisms involved in triggering escape and locomotory jumps may be different, with a combination of proprioceptive and exteroceptive inputs being involved in the former, but only proprioceptive input being responsible for triggering the latter. The present findings suggest that this may not be true, and that perhaps, in both cases, proprioceptive feedback alone might ultimately be responsible for producing trigger activity. This proposal is considered in the following chapter by examining the way in which specific hindleg proprioceptors may contribute to the generation of the motor program and its trigger signal.

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V. THE ROLE OF PROPRIOCEPTIVE INPUT IN THE JUMP SYSTEM

A. INTRODUCTION

Before a locust can kick or jump, it must first flex its hind tibiae fully about the femoral-tibial joint (Bässler 1968). Continued flexor activity then holds the tibia in this position while the extensor muscle undergoes a prolonged isometric contraction (Brown 1967; Godden 1975; Heitler and Burrows 1977a). As extensor tension increases during this period of co-contraction, energy is stored in elastic cuticle of the distal femur and in the extensor apodeme (Bennet-Clark 1975). The jump or kick is finally triggered when a sudden relaxation of the flexor muscle permits most of the stored energy to be converted into kinetic energy as the hind tibiae rapidly extend. In Chapter 2, it was suggested that a system of unidentified metathoracic interneurons, for convenience termed the central trigger system, may be responsible for producing this flexor muscle relaxation. The system apparently achieves this by controlling the activity of a number of other neurons, including the M-neurons (Pearson et al. 1980), which are directly involved in terminating flexor activity at the end of the motor program (Chapter 3). However, it is not yet known how this hypothetical central system is itself activated to trigger kicks and jumps.

As described in the preceding chapter, the notion that triggering results from a summation of exteroceptive and proprioceptive inputs at some central site does not appear to be correct because sudden exteroceptive stimuli delivered during the co-contraction phase failed to elicit trigger activity. There is no question, however, that proprioceptive information is vital for the performance of kicks and jumps. If an obstruction prevents the hind tibiae from being fully flexed, a locust can no longer jump (Brown 1967, Bässler 1968). Jumping is also prevented by destruction of the hindleg chordotonal organs (COs), which encode, among other things, femoral-tibial joint position (Usherwood, Runion and Campbell 1968). Since a similar result is achieved by severing the organ's main apodeme (Bässler 1968), an operation which specifically abolishes responses in the range of joint flexion (Zill 1985), it has been

suggested that it is signals from the CO during tibial flexion that are critical for locusts to be able to kick and jump. How, then, might the CO contribute to the triggering process? Zill (1985) found that when the leg is held in the fully flexed position, the CO responds to distortion of the proximal tibia caused by contractions of the extensor muscle. Thus, one suggestion is that the organ may indirectly monitor extensor muscle tension resulting from activity of the fast extensor (FETi) motoneuron during the motor program. However, Heitler and Bräunig (1988) have recently demonstrated that the main features of the kick program, including the sudden termination of flexor activity during the trigger phase, can occur in the complete absence of FETi activity. This indicates that the generation of the trigger signal cannot be dependent upon extensor muscle tension and, therefore, raises the question of whether the CO might be involved by signalling some aspect related to leg flexion during the motor program. Indeed, this idea had been suggested by Bässler's study twenty years earlier.

Another proprioceptor that has been implicated in the control of jump triggering, and whose activation is dependent on hindleg flexion, is the lump receptor (Heitler and Burrows 1977b). Due to its particular location in the distal femur, this receptor is only excited when the tibia is fully flexed and there is tension in the flexor tendon. This is precisely the situation that exists during the co-contraction phase. Afferents from this receptor run in the lateral nerve and when this is cut, it is more difficult to elicit kicks or jumps from an animal. Periods of co-contraction still occur, but these are not usually terminated by trigger activity. Furthermore, mechanical stimulation of the lump receptor, or electrical stimulation of the lateral nerve, can both cause inhibition of flexor motoneurons. However, as with the CO, the detailed mechanisms by which the lump receptor might trigger kicks and jumps are not known.

The aim of the present investigation, then, was to determine whether these proprioceptors are important for generating the trigger phase of the motor program, and if so, whether the peripheral signals they provide are of a phasic or tonic nature. These questions were addressed by assessing the effects of nerve transection or receptor ablation on

the motor program, and by recording from, and stimulating, sensory nerves during the performance of bilateral kicks. The results indicate that the lump receptor plays no role in the triggering process, but that the CO is of vital importance. Furthermore, CO input is essential for co-contraction to occur. It appears that tonic feedback from the CO encoding full tibial flexion is involved in producing both of these phases of the motor program.

B. MATERIALS AND METHODS

Details of the dissection and of techniques for both intracellular and electromyographic recording were as described in the preceding chapter. Additional techniques were as follows.

Nerve Recording and Stimulation

To expose the lateral nerve, one hindleg was manipulated so that it lay in a horizontal plane, perpendicular to the animal's long axis and with its medial (inner) surface facing upwards. A small piece of cuticle spanning the prominent ridge which forms the ventro-medial margin of the femur was removed. The exact location of the window was not critical. Saline was not used to irrigate the preparation because it often caused the leg to autotomize or the extensor muscle to contract powerfully, snapping its tendon. The lateral nerve was quickly placed on a pair of 75 µm silver wire hook electrodes and insulated with silicon grease.

Ideally, recordings of CO afferent activity would be made from the chordotonal nerve which joins the organ to nerve 5b1, however, this was difficult to do without causing some injury to the leg which then prevented it from kicking. Consequently, recordings were made from the whole nerve 5b1 some 8 mm proximal to the CO. Any activity of tibial afferents in these recordings was eliminated by macerating the inside of the tibial stump with a blunt probe. The leg was positioned as for lateral nerve recording and a small window was cut in the femoral cuticle. A fine steel hook was used to search for nerve 5b1 in the space between

the flexor and extensor muscles.

Recordings from the lateral nerve and nerve 5b1 were never free of contamination from flexor and extensor muscle potentials. This situation was improved somewhat by high-pass filtering (using a Krohn-hite 3700 filter) with a low frequency cut-off of 500-600 Hz. The nature of the afferent spikes in these recordings was not greatly affected by the filtering process.

The timing of nerve stimulation during co-contraction was controlled by the digital device described in the preceding paper. In most cases, the stimuli were 200 Hz, 60 ms trains of 0.2 ms duration pulses. Trains applied to nerve 5b1 were usually 1.5 V, whereas more intense stimulation (3 V) was used for the lateral nerve.

Nerve and Receptor Apodeme Transection

The lateral nerve could easily be cut after exposing it as described above. To sever the CO nerve, a narrow window, 2-3 mm long, was cut in the pale colored, dorso-medial cuticle of the femur, just proximal to the dark, heavily sclerotized region. For the leg to be able to kick, it was critical that this window be cut between the longitudinal strengthening ribs. The tracheae under the cuticle were removed. Moving the tibia into the fully extended position drew the extensor tendon ventrally and provided a clearer view of the CO, adjacent to the lateral wall of the femur. The CO nerve could then be cut. Because of the small aperture of the window it was sometimes difficult to determine whether this operation had been performed successfully, and so to ensure this, the femur was fully dissected following each experiment. In other experiments, the main apodeme of the CO was severed using the technique described by Bässler (1979), although no attempt was made to reattach this apodeme at another site.

In all transection experiments, EMGs were recorded during hindleg kicks following the operation to expose the particular nerve or apodeme, but prior to cutting it, and then again after the cut was made. In addition, behavioral observations and EMG recordings were made

of animals whose CO apodemes in one or both legs had been severed several days earlier. Control animals had undergone the same operation, but their receptor apodemes had been left intact.

C. RESULTS

The Role of the Lump Receptor

As a first step towards assessing the possible role of the lump receptor in triggering kicks, flexor and extensor EMGs were recorded from a single leg before and after transecting the lateral nerve. The opposite hindleg was prevented from kicking to ensure that contralateral sensory feedback could not influence the recorded motor pattern. As reported by Heitler and Burrows (1977b), locusts were still able to kick after input from the lump receptor had been abolished. However, contrary to their observations, lateral nerve transection did not alter the ease with which kicks could be elicited. An intact, restrained animal could usually be induced to kick 10-20 times, and this was also true for animals whose lateral nerves had been severed. Moreover, the operation had no apparent effect on the duration of the entire motor program, or of its separate phases. Examples of patterns of motor activity recorded before and after nerve transection are shown in Fig. 5.1. Clearly, these patterns are very similar.

Although it is obvious from these findings that the lump receptor is not critical for the performance of kicks, it may nevertheless contribute to the generation of the trigger signal. Heitler and Burrows (1977b) reported that the lump receptor is activated when the leg is in the fully flexed position and there is tension in the flexor tendon. Not surprisingly, then, recordings from the lateral nerve during kicks revealed that afferent activity commences during the initial flexion period and continues throughout the co-contraction phase (Fig. 5.2). There was no obvious phasic discharge corresponding to the moment of triggering, as indicated by the time of flexor motoneuron shutdown. The intense burst of large amplitude afferent spikes following the termination of flexor activity was caused by leg extension during

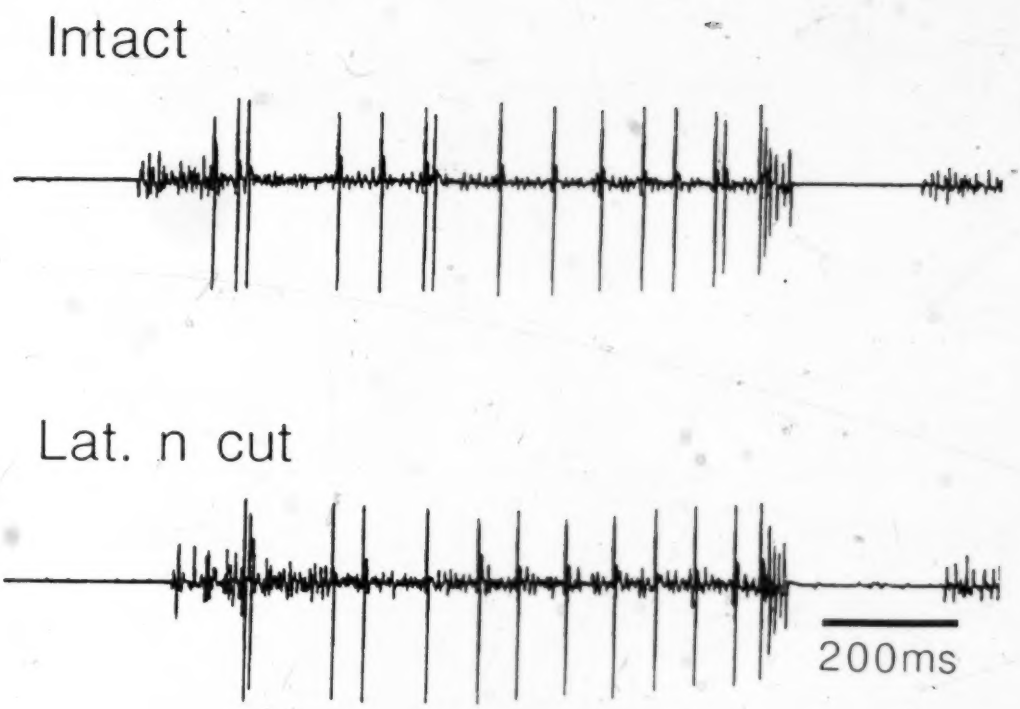

Fig. 5.1. Transection of the lateral nerve has no effect on the kick motor program. Electromyographic recordings from the flexor and extensor tibiae muscles of one hindleg before (top trace) and after (bottom trace) the lateral nerve was severed. The large amplitude spikes are due to activity of the fast extensor motoneuron; the smaller spikes are caused by activity of the various flexor motoneurons. Patterns of motor activity similar to those during normal kicks occurred following elimination of lump receptor input.

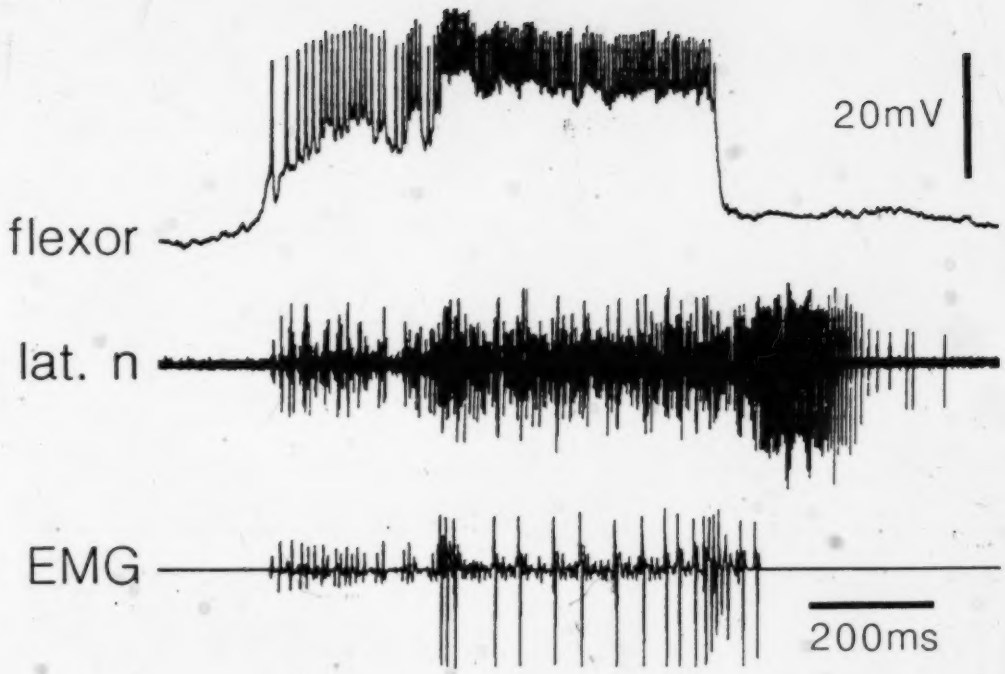

Fig. 5.2. Afferent activity recorded from the lateral nerve during a kick. Top trace - intracellular recording from a flexor motoneuron innervating the left leg; second trace - extracellular recording of afferent activity in the left lateral nerve; bottom trace - combined flexor and extensor EMG recording from the left leg. Some pick-up of muscle potentials is evident in the lateral nerve recording. The spikes at the beginning of the initial flexion phase, and some of the larger amplitude spikes during co-contraction are due to flexor and extensor activity, respectively. Note that tonic afferent activity occurs throughout the co-contraction phase, but there is no obvious phasic discharge at, or just prior to, the instant of flexor motoneuron repolarization.

the kick and was most likely due to receptors located in the suspensory ligaments of the joint (Coillot and Boistel 1969). These results suggest that if the lump receptor does play a role in the triggering process, it probably does so by providing tonic feedback during co-contraction.

Another strategy used to assess whether lump receptor input is involved in producing trigger activity was to apply brief, high frequency stimulus trains to the lateral nerve in one hindleg during the co-contraction phase of bilateral kicks. The rationale behind this was that the excitation produced in the central nervous system (CNS) by the electrical stimulation should summate with that caused by the ongoing afferent activity and perhaps trigger a kick prematurely. During normal bilateral kicks, the shutdown of flexor activity during the trigger phase occurs almost simultaneously in both hindlegs, and the left and right legs extend in near synchrony. It can be seen in Fig. 5.3 that stimulating the left lateral nerve during co-contraction prematurely terminated the activity of all ipsilateral flexor motoneurons (as judged by the intracellular recording and by the absence of flexor spikes in the left leg EMG during and after the pulse train), but did not influence the motor program in the contralateral leg. Co-contraction continued in the opposite leg as usual. Consequently, lateral nerve stimulation produced a marked discrepancy in the timing of extension of the two legs. As was noted in Chapter 3, the prematurely triggered leg subsequently displayed a burst of extensor activity corresponding to the terminal burst in the unaffected, contralateral leg.

During the trigger phase of normal kicks, the membrane potential of flexor motoneurons is rapidly repolarized to its resting level. However, it was consistently noted that during kicks evoked by lateral nerve stimulation the flexors exhibited a slight maintained depolarization after the pulse-train, lasting until the time the opposite leg was triggered (Fig. 5.3). This suggested that some aspect of the experimentally-evoked trigger activity was not natural. Since the M-neurons are known to discharge powerfully during the trigger phase (Chapters 2,3), it was possible to determine whether the flexor repolarization produced by lateral nerve stimulation was due to the normal activation of the proposed central trigger system simply by observing the activity of the M-neurons at the time of stimulation.

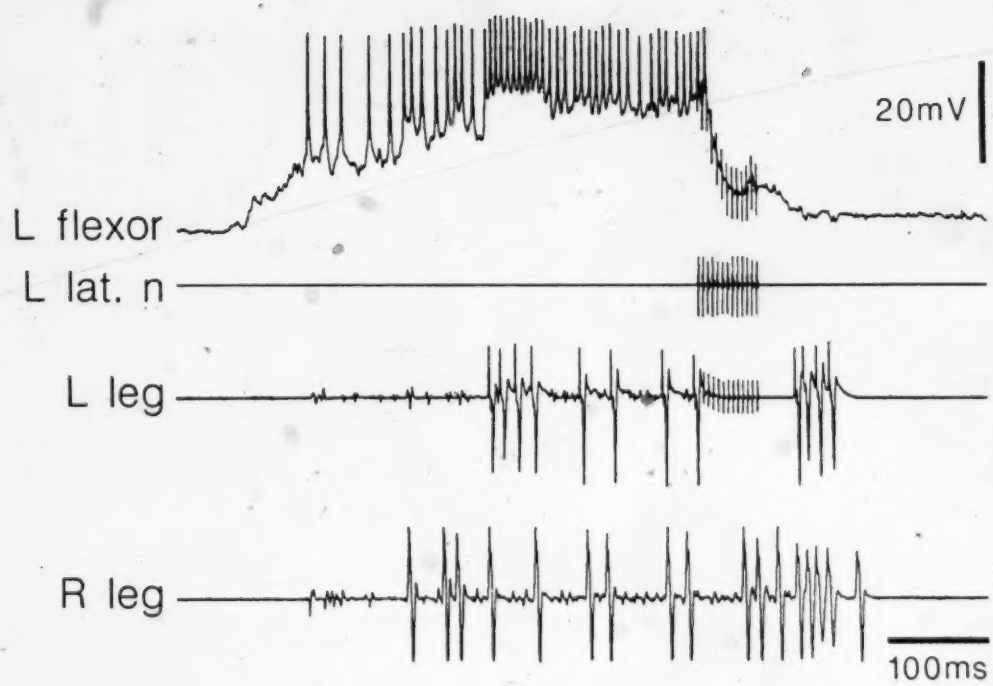

Fig. 5.3. Premature flexor repolarization produced by lateral nerve stimulation during co-contraction. Top trace - intracellular recording from a flexor motoneuron innervating the left leg; second trace - 3 V, 200 Hz pulse train applied to the left lateral nerve; lower traces - left and right leg EMGs. The pulse train during the co-contraction phase caused the sudden repolarization of the flexor motoneuron and silenced activity in all other ipsilateral flexors as well (as is evident by the absence of flexor spikes in the left leg EMG). Flexor and extensor activity in the right leg was unaffected by the pulse train. Note the small maintained depolarization in the flexor motoneuron following the stimulus train.

Figure 5.4 shows two examples of M-neuron recordings during kicks in which stimulation of the left lateral nerve interrupted the co-contraction phase in the left leg and caused it to extend prematurely. In each case, the pulse train failed to excite the ipsilateral M-neuron, either directly, or indirectly via activation of the hypothetical trigger system. The slight depolarization in M following the stimulus train in Fig. 5.4A and the single spike in Fig. 5.4B were due to reflex excitation caused by the leg's extension, and were not a direct result of the stimulus train. After the leg extended prematurely, the M-neuron discharged a weaker than normal burst of action potentials at the normal time of trigger activity in the opposite leg. This same phenomenon was observed in the study described in Chapter 3. The finding that the M-neurons were not activated during these experimentally-evoked kicks implies that the triggering produced by lateral nerve stimulation is an artificial one. The lump receptor, therefore, probably plays no role in the process by which kicks and jumps are normally triggered.

How, then, did lateral nerve stimulation bring about the premature repolarization of ipsilateral flexor motoneurons during co-contraction? The answer to this is apparent in Fig. 5.5. When pulse trains which were able to elicit premature leg extension during co-contraction were applied to the lateral nerve in the quiescent animal, they usually produced a distinct hyperpolarization of ipsilateral flexors, but, again, typically did not evoke action potentials in either M-neuron (Fig. 5.5A). Similarly, prolonged stimulation caused a maintained hyperpolarization of flexor motoneurons, identical to that reported by Heitler and Burrows (1977b), but only a transient subthreshold depolarization of the M-neurons (Fig. 5.5B). Thus, the flexor inhibition produced by this electrical stimulation must have been due to direct inhibitory pathways (independent of interneurons in the trigger system) that exist between afferents in the lateral nerve and ipsilateral flexor motoneurons.

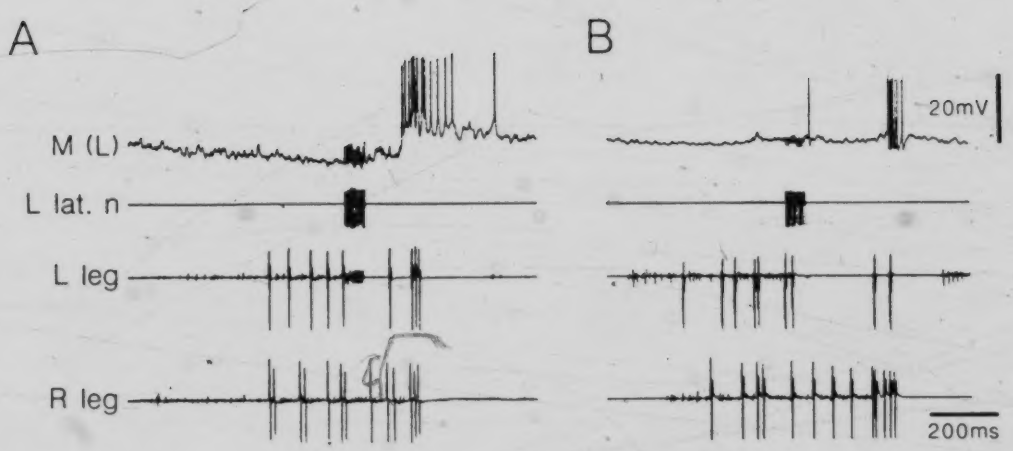

Fig. 5.4. Lateral nerve stimulation causing premature leg extension during co-contraction does not activate the ipsilateral M-neuron. Top traces - intracellular recordings from M-neurons which inhibit left leg flexors; second traces - stimulus trains (3 V, 200 Hz) applied to the left lateral nerve; lower traces - left and right leg EMGs. The recording in A was made from the medial processes of the M-neuron. A lateral branch recording is shown in B. In each example, the pulse train terminated co-contraction in the left leg and caused premature extension, but did not evoke a discharge of action potentials in the M-neuron. Note that the motor program in the right leg continued well beyond the time of lateral nerve stimulation.

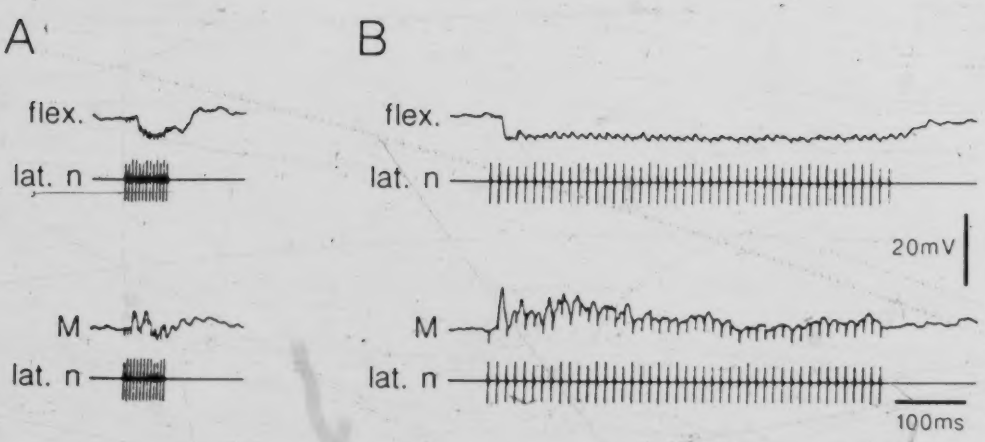

Fig. 5.5. Effects of lateral nerve stimulation on ipsilateral flexor motoneurons and M-neurons in the quiescent animal. Top traces - intracellular recordings from a left leg flexor motoneuron (*flex.*) or the M-neuron which inhibits left leg flexors (*M*); bottom traces - 3 V pulse trains applied to the left lateral nerve. The M-neuron and flexor recordings were made in different animals, however, in each animal pulse trains of this intensity were able to cause premature extension of the left leg during co-contraction. A Brief 200 Hz stimulation of the lateral nerve usually hyperpolarized ipsilateral flexors, but produced only a combination of weak excitation and inhibition of the M-neuron. B Prolonged 80 Hz stimulation produced a maintained hyperpolarization of flexor motoneurons in which each stimulus pulse was followed by a small depolarization. The same stimulation evoked only a short-lived, subthreshold depolarization in the M-neuron.

The Role of the Chordotonal Organ

Bässler (1968) reported that animals whose CO apodemes have been severed are still able to flex their tibiae normally, but can no longer jump. This was thought to be because the jump is not triggered. It would, therefore, be expected that co-contraction would still take place in the operated leg, but this would not be terminated by trigger activity. Consequently, excitation of the femoral muscles should gradually diminish. To test this prediction, EMGs were recorded from both hindlegs during normal bilateral kicks and during kicks elicited after the CO apodeme in one leg had been transected. Consistent with the earlier findings, once the apodeme had been cut the operated leg was no longer able to kick. However, this was not simply because the trigger phase of the motor program was absent, but because the entire co-contraction phase did not occur (Fig. 5.6). The operated leg was often moved into the fully flexed position, but simultaneous fast extensor activity never took place. In some kicks, flexor activity in the operated leg continued throughout the period of co-contraction in the contralateral hindleg, until just before the time of triggering. On other occasions, no flexor activity was observed in the operated leg during the performance of the contralateral kick motor program. A consistent feature, though, was that one to six FETi spikes occurred in the operated leg at a time roughly coincident with the terminal extensor burst in the intact leg. This same extensor excitation was evident following premature leg extension in Figs. 5.3 and 5.4, and is thought to reflect a central, bilateral output to the metathoracic FETi motoneurons at the end of the motor program (Chapter 2). The significance of this extensor muscle activation is that it caused the operated leg to extend in approximate synchrony with the intact, kicking leg, thus giving the illusion of a normal bilateral kick. When this extensor burst was strong (e.g. 3-6 spikes), this illusion was quite convincing.

Transection of the CO apodeme in one leg did not appear to influence the duration of any aspect of the kick motor program in the contralateral leg. Although it may appear in Fig. 5.6 that the operation caused a prolongation of the initial flexion phase, brief periods of initial flexion also occurred in this animal after the apodeme had been cut. The durations of

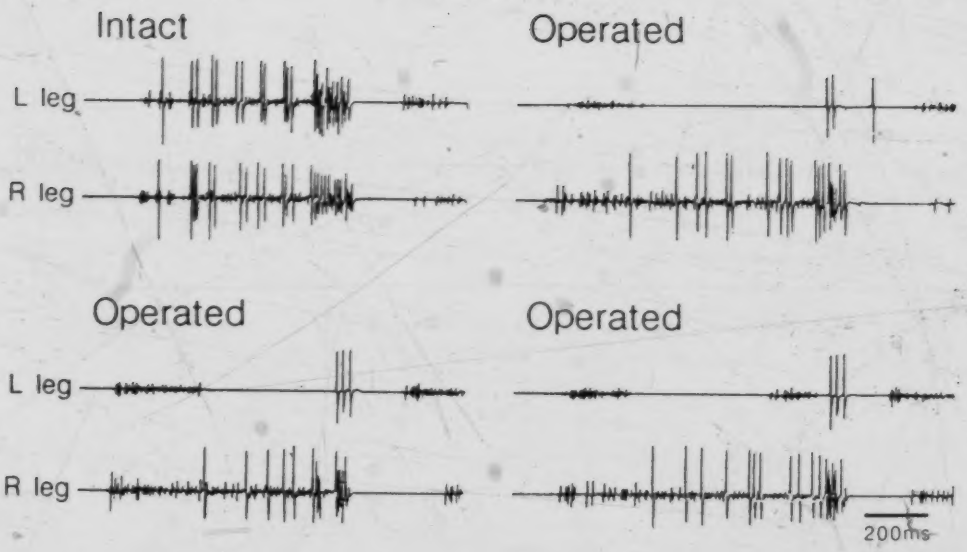

Fig. 5.6. Severing the receptor apodeme of the chordotonal organ (CO) prevents initiation of the co-contraction phase in the ipsilateral leg. Examples of EMG activity recorded from the left (top traces) and right (bottom traces) hindlegs before (*intact*) and after (*operated*) transecting the left CO apodeme. Once the apodeme was cut, co-contraction of the flexor and extensor muscles in the left leg could not be initiated, even though normal co-contraction occurred in the contralateral leg. The left leg was often fully flexed before the kick of the right leg. It also extended in near synchrony with the intact right leg due to a variable number of FETi spikes which occurred at about the time of trigger activity. Three 'operated' examples are shown in order to emphasize the consistent occurrence of these FETi spikes in the ipsilateral leg.

the different phases of the motor program in each animal are normally highly variable (see also Heitler and Bräunig 1988), and the examples shown in Fig. 5.6 were selected only to demonstrate that normal episodes of co-contraction (complete with their terminal extensor bursts) can occur in a leg following the transection of the contralateral CO apodeme. Severing the CO nerve, which eliminated all afferent input from the CO, also prevented kicking by abolishing the co-contraction phase. Indeed, the effects of this operation were identical to those described for section of the CO apodeme.

Given the drastic alterations of the motor program in the ipsilateral leg produced by cutting the CO apodeme, Bässler's (1979) conclusion that this operation only temporarily abolishes an animal's ability to jump and kick seemed surprising. To test this, the receptor apodemes of one or both legs of locusts were cut, and observations of the jumping and kicking performance of these animals were made several days later. Control animals were operated on in the same way but their CO apodemes were left intact. As Bässler had noted, locusts with one or both CO apodemes severed still appeared to jump. However, such behavioral observations were misleading because when these animals were later restrained and EMG recordings made from both hindlegs during bilateral kicks, it was discovered that here, also, the "kicks" of the operated legs were never preceded by flexor and extensor co-contractions. Rapid leg extension was produced only by brief bursts of FETi spikes. In those cases in which only one apodeme had been cut, this extensor burst coincided with the terminal burst in the intact leg, just as in the examples shown in Fig. 5.6. When the apodemes of both COs had been severed (preventing co-contraction in either leg) bilateral tibial extension could still sometimes occur due to a near synchronous burst of extensor activity in the two hindlegs. It is not known whether this extensor excitation arises from the same source that is thought to produce the terminal extensor burst during the normal kick or jump motor program (Chapter 2). The pattern of motor activity recorded from the operated legs of control animals was normal.

Although these findings indicate that chordotonal input is essential for the initiation of co-contraction, they do not reveal whether the CO is also important for the generation of the trigger phase, since this is a separate stage of the motor program which can only occur after co-contraction has taken place. To answer this question, then, it was necessary to record from, and to stimulate, CO afferents during the performance of kicks. Extracellular recordings from CO afferents during kicks typically show intense activity at the onset of the initial flexion phase of the motor program (Fig. 5.7A). A phasic discharge occurred as the tibia was moved into the fully flexed position, and tonic activity then continued for the remainder of the flexion phase and throughout the co-contraction phase. In Fig. 5.7B the rapid extension of the leg prior to the start of the kick motor program produced afferent activity that did not completely subside before the initial flexion phase commenced. During co-contraction, bursts of afferent spikes occurred in response to contractions of the extensor muscle (particularly evident in Fig. 5.7B), presumably as a result of distortion of the proximal tibia and displacement of the receptor's apodeme (Zill 1985). Although the prominent phasic discharge at the conclusion of the motor program in Fig. 5.7A appears as if it might represent a peripheral trigger signal, it is quite obvious in Fig. 5.7B that this discharge occurs after flexor motoneuron activity has been terminated. It must, therefore, have been a response to the leg's ballistic extension during the kick. Prior to the moment of triggering (i.e. flexor shutdown) no other phasic afferent discharge is apparent in these recordings, suggesting that if the CO does play a role in generating the trigger phase, then it may be the activity of the organ's tonic units that is responsible.

To assess whether CO afferents are able to evoke trigger activity, nerve 5b1, just proximal to the organ, was electrically stimulated with brief, high frequency pulse trains during the co-contraction phase of bilateral kicks. Figure 5.8 shows two examples of recordings made from ipsilateral flexor motoneurons during such stimulation. In both cases, the pulse train caused the motoneuron to rapidly repolarize, but had no immediate effect on the contralateral flexors, as can be seen from the continuing flexor activity in the right leg

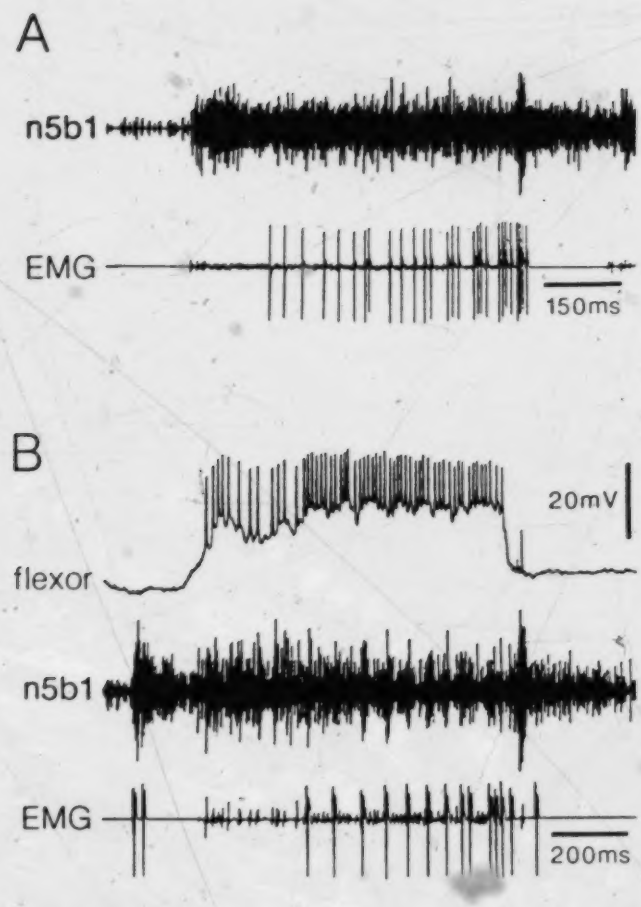

Fig. 5.7. Chordotonal afferent activity during kicks. Bottom traces - flexor and extensor EMGs recorded from the left leg; top trace in **A** and middle trace in **B** - extracellular recording of afferent activity from the left nerve 5b1, just proximal to the CO; top trace in **B** - intracellular recording from a left leg flexor motoneuron. The initial movement of the leg at the start of the motor program (or preceding it in **B**) caused a phasic burst of afferent activity. Sustained firing of tonic units occurred once the leg reached full flexion, but superimposed on this are bursts of afferent spikes produced in response to extensor muscle contractions (especially evident in **B**). The phasic chordotonal discharge close to the end of FETi activity occurred after the repolarization of the flexor motoneuron (i.e. after the trigger phase) and was due to the leg's rapid extension during the kick. No obvious phasic discharge is visible prior to the kick being triggered.

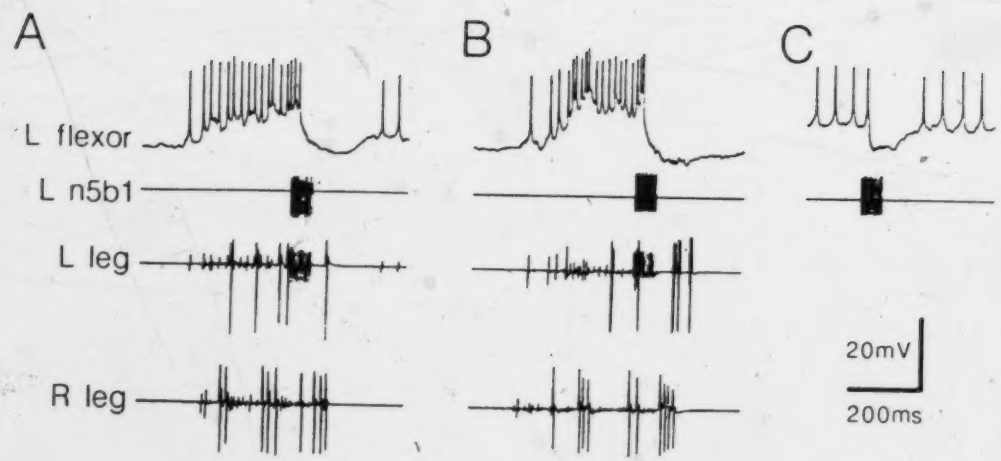

Fig. 5.8. Premature repolarization of ipsilateral flexors produced by chordotonal stimulation during co-contraction. Top traces - intracellular recordings from flexor motoneurons innervating the left leg; second traces - 1.5 V, 200 Hz stimulation of the left nerve 5b1; bottom traces in **A,B** - left and right leg EMGs. **A,B** are examples from different animals. In each case, the pulse train caused the sudden termination of activity in the left leg flexors (see intracellular and EMG recordings) but did not produce an immediate cessation of flexor activity in the right leg. Note that the membrane potential of the flexor motoneuron returned to its resting level following the stimulus. **C** The same stimulation in a quiescent animal caused a hyperpolarization of ipsilateral flexor motoneurons that had been depolarized by the injection of constant current. The latency of this effect was the same as for the flexor repolarization evoked during co-contraction (cf. **A,B**).

myogram. If a flexor motoneuron in a quiescent animal was injected with constant depolarizing current, such that it was firing tonically, stimulation of the ipsilateral nerve 5b1 also caused a strong repolarization of its membrane potential (Fig. 5.8C). The latency of this inhibition in the quiescent animal was identical to that for the flexor repolarization produced by the stimulation during co-contraction. Depending on the preparation, this latency was between 18 and 20 ms. Thus, as was the case for the lateral nerve, it is probable that the premature triggering of the ipsilateral leg caused by nerve 5b1 stimulation was a directly-evoked reflex effect and was not due to activation of the system that normally triggers kicks. This conclusion is further supported by the observation that FETi activity in the ipsilateral leg was merely interrupted by the pulse train, and the usual terminal extensor burst close to the time of triggering did not occur.

A key observation during these experiments was that, although chordotonal stimulation caused the early release of the ipsilateral leg, on no occasion was there a large discrepancy in the timing of left and right leg extension. This can be judged approximately by the relatively short duration between the time of stimulation of the left nerve 5b1 and the end of right leg EMG activity in the two examples shown in Fig. 5.8. This situation contrasts markedly with the "triggering" produced by lateral nerve stimulation in which the affected leg could sometimes be caused to extend several hundred milliseconds before the kick of the contralateral leg (see Fig. 5.4). This suggested that stimulation of the CO afferents in one leg could somehow influence the timing of trigger activity in the opposite leg. Such an effect is clearly demonstrated in Fig. 5.9, which illustrates the relationship between the time of chordotonal stimulation during the co-contraction phase and the change in duration of co-contraction (or, more accurately, the duration of FETi activity) in the contralateral leg. Stimulation early in the co-contraction phase caused a distinct shortening of co-contraction in the opposite leg, as compared to an average duration calculated for normal kicks in the same animal. Stimulation late in the co-contraction phase also caused shortening, but to a much lesser extent. For three of the four animals in which these values were measured, the slopes of

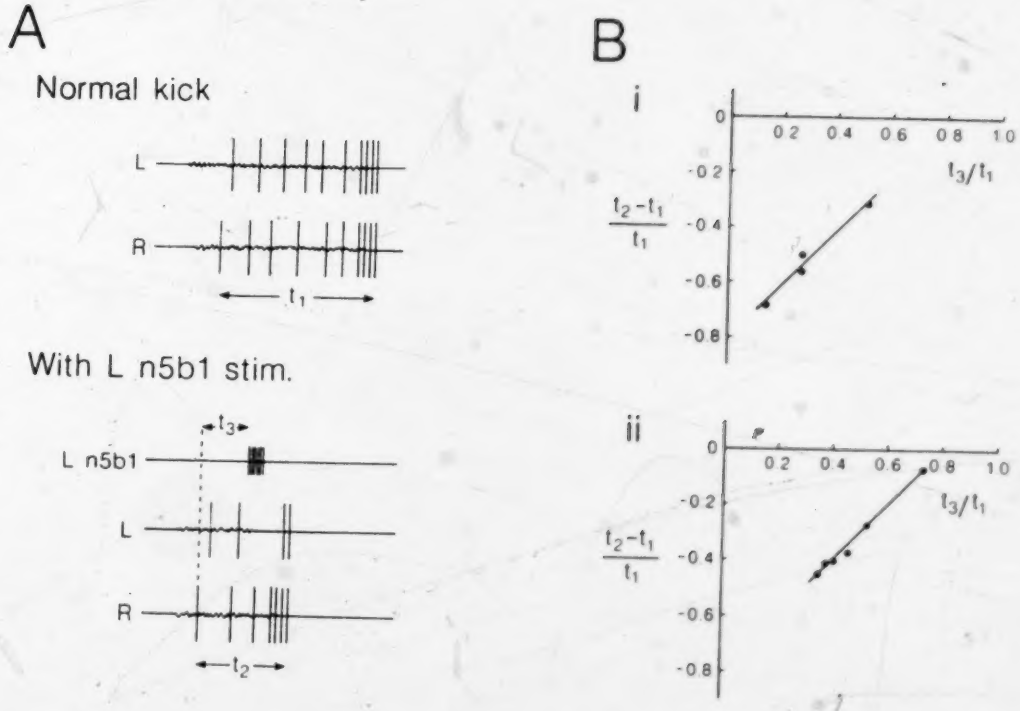

Fig. 5.9. Chordotonal stimulation terminates the co-contraction phase in the contralateral leg. **A** Diagram to illustrate the variables measured from recordings of EMG activity during kicks with, and without, sensory stimulation. **B** Relationship between the time a 1.5 V, 200 Hz pulse train is applied to the left nerve 5b1 and the change in duration of FETi activity in the right leg. Data from two different animals are shown (i,ii). For each plot, both variables were normalized by dividing by the average duration of extensor activity (t_1) during normal kicks in that animal.

the regression lines fitted to the data did not differ significantly from 1.0 ($p>0.05$). The data from two of these animals are illustrated (Fig. 5.9B). That the slope of this relationship is close to 1.0 implies that there is a relatively fixed interval between the time of nerve 5b1 stimulation and the conclusion of the motor program in the contralateral leg (i.e. t_2-t_3 is approximately constant), regardless of when the pulse train is applied during co-contraction. This interval had a finite value and consequently the X and Y intercepts of the regression lines were not +1.0 and -1.0, respectively. In 35 kicks from eight animals, the average delay between the stimulus onset and the end of contralateral EMG activity was 103.7 ms (S.D.=16.8 ms). It should be emphasized that had chordotonal stimulation been without effect, the data points in these plots would lie along the abscissa.

From these results it is apparent that chordotonal stimulation exerts a strong influence on the motor program in the contralateral leg. A number of observations indicate that this influence may be a reflection of the natural triggering process. Firstly, a terminal burst of extensor muscle spikes was always present in the EMG of the contralateral leg after co-contraction had been curtailed by stimulating nerve 5b1 (Fig. 5.8, see Figs. 5.10-12). This suggests that the motor program in the opposite leg had been brought to its normal close and was not somehow interrupted by the pulse train. Consistent with this, extensor spikes also occurred in the ipsilateral, prematurely triggered leg at the time of trigger activity in the opposite leg (Fig. 5.8), although the timing of this EMG activity relative to the contralateral burst was sometimes quite variable. The most telling finding, though, was that chordotonal stimulation during co-contraction always fully repolarized ipsilateral flexor motoneurons (Fig. 5.8). There was no maintained depolarization after the pulse train like that observed in flexors following lateral nerve stimulation (see Fig. 5.3). Thus, although some reflex pathway may have caused the initial shutdown of ipsilateral flexor activity, complete repolarization of the flexor motoneurons after the nerve 5b1 pulse train was probably due to the subsequent activation of the central trigger system.

If nerve 5b1 stimulation during the co-contraction phase does produce normal trigger activity, this should be reflected by the delayed termination of contralateral flexor activity and by the powerful activation of the two M-neurons, with both these events having similar latencies. This was found to be true. Figure 5.10 shows that chordotonal stimulation was followed after some delay by the sudden shutdown of the contralateral flexor motoneurons. The latency between the first pulse in the train and the onset of the steep repolarization in these flexors was not constant, but in the three animals in which such recordings were made it was always in the range of 40-60 ms. This was much longer, and more variable, than the 18-20 ms required for the termination of ipsilateral flexor activity (see Fig. 5.8). Also, unlike what was observed in ipsilateral flexor motoneurons, these same pulse trains had no effect on tonically active contralateral flexors in the quiescent animal (Fig. 5.10C). Therefore, inhibitory pathways between chordotonal afferents and the flexor motoneurons of the opposite leg do not exist. These observations are all consistent with the idea that the termination of activity in the contralateral flexors during co-contraction was not due to a reflex inhibition, but rather to excitation of the trigger system.

Recordings from seven different M-neurons made while nerve 5b1 was stimulated during co-contraction revealed that both members of the pair were always strongly depolarized soon after the onset of the pulse train (Figs. 5.11, 5.12). However, the rising phases of these depolarizations were not as rapid as is usually seen in an M-neuron because each typically commenced with a phasic subthreshold depolarization, followed by a transient repolarization and then further depolarization. These synaptic inputs prior to the burst of action potentials were very similar, in both form and latency, to the direct inputs the M-neurons received from pulse trains in the quiescent animal (Figs. 5.11C, 5.12C). This suggested that the depolarization produced in M by stimulation of CO afferents may have been due to a combination of directly-evoked inputs and input from the central trigger system. The onset of intense spike activity in these neurons occurred 40-60 ms after the start of the stimulus train, matching the range of latencies measured for the steep repolarization of contralateral flexors

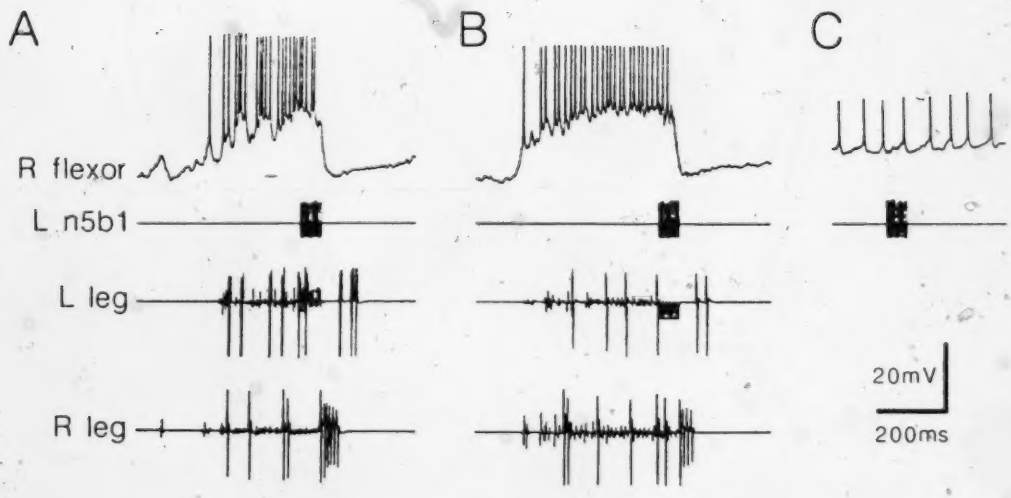

Fig. 5.10. Chordotonal stimulation during co-contraction causes the delayed repolarization of contralateral flexor motoneurons. Top traces - intracellular recordings from right leg flexor motoneurons; second traces - 1.5 V, 200 Hz pulse trains applied to the left nerve 5b1; bottom traces in A,B - left and right leg EMGs. A,B are recordings from different animals. In each example, there is a short delay between the time of stimulation and the onset of rapid repolarization in the flexor. Note that the motor program in the right leg concluded with its usual extensor burst. C Identical stimulation in the quiescent animal had no effect upon contralateral flexors depolarized by current application.

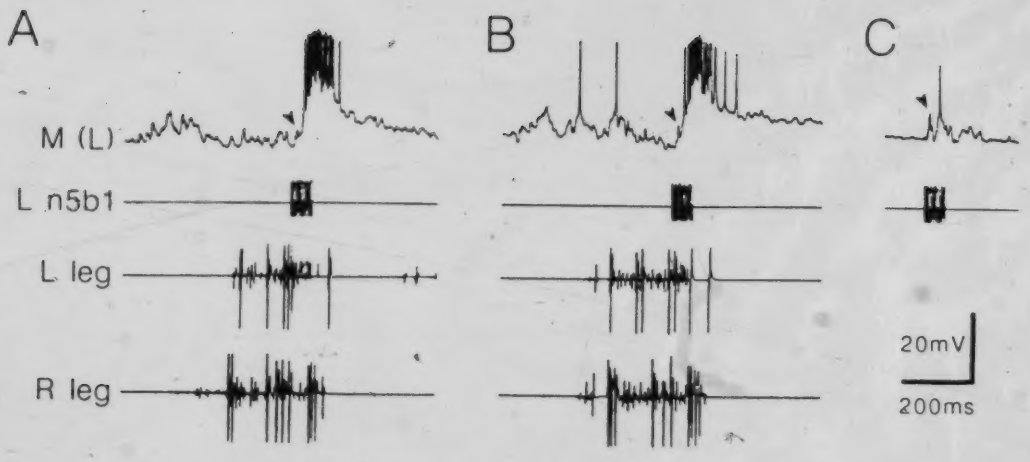

Fig. 5.11. Chordotonal stimulation during co-contraction activates the ipsilateral M-neuron. Top traces - intracellular recordings from the M-neuron which inhibits left leg flexors; second traces - 1.5 V, 200 Hz stimulation of the left nerve 5b1; bottom traces in **A,B** - left and right leg EMGs. All recordings are from the same animal. **A,B** Stimulating nerve 5b1 during co-contraction caused a powerful depolarization in the M-neuron, with high frequency spike activity occurring after a short delay. The rising phase of M's discharge displayed synaptic potentials similar to those evoked directly by pulse trains in the quiescent animal (**C**). The first of these is visible in each example (arrowhead). The subsequent suprathreshold depolarization evident in the resting animal was not always discernible during co-contraction because it apparently merges with the M-neuron's strong depolarization. Note the terminal extensor burst at the end of the motor program in the contralateral leg.

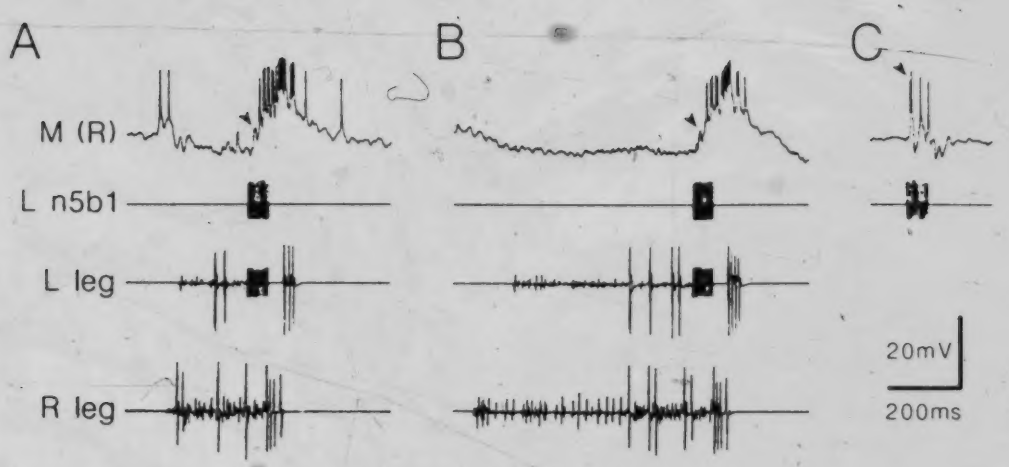

Fig. 5.12. Chordotonal stimulation during co-contraction activates the contralateral M-neuron. Top traces - intracellular recordings from the M-neuron which inhibits right leg flexors; second traces - 1.5 V, 200 Hz pulse trains applied to the left nerve 5b1; bottom traces in A,B - left and right leg EMGs. All recordings are from the same animal. A,B The M-neuron was strongly depolarized shortly after the onset of chordotonal stimulation during co-contraction. The trigger discharges in the neuron were preceded by synaptic inputs evoked directly by the pulse trains. These inputs during co-contraction appeared to be shunted versions of the potentials produced by stimulation in the quiescent animal (C, compare the amplitudes of the initial depolarizations indicated by the arrowheads). Again, note that the motor program in the right leg concluded with an extensor burst.

(Fig. 5.10).

Although it may appear by comparing the intensity of the discharges in Figs. 5.11 and 5.12 that chordotonal stimulation exerted a weaker influence on the contralateral M-neuron, this was not so. In other animals, the contralateral M discharged as powerfully after the pulse train as it did during normal kicks. In the animal from which the recordings shown in Fig. 5.12 were made, the effect of stimulating nerve 5b1 was to produce some strong inhibitory potentials (in addition to the weak excitation, Fig. 5.12C). This directly-evoked inhibitory input probably counteracted the depolarizing input to the neuron arising from the trigger system and consequently lessened the intensity of the neuron's initial discharge. Clearly though, the amplitude of the underlying depolarization during this burst of spikes was as large as that evoked in the ipsilateral M-neuron by stimulating the chordotonal afferents (cf. Fig. 5.11).

On two occasions in one animal, stimulating nerve 5b1 with pulse trains of an intensity that had earlier been sufficient to produce trigger activity failed to terminate the co-contraction phase in the contralateral leg after the usual delay. It is significant that on both occasions normal co-contraction had not been initiated in the stimulated leg and there had been unusually long periods of initial flexion (Fig. 5.13A). The pulse train still caused the shutdown of ipsilateral flexor motoneurons, but this was only transient (see left leg EMG). Corresponding to the stimulus train's failure to evoke trigger activity, the recording from the M-neuron (contralateral in this case) reveals only the potentials produced as a direct result of chordotonal stimulation, but not the powerful excitation normally associated with triggering. This occurred later at the normal conclusion of the motor program in the right leg. Furthermore, the direct inputs to M during co-contraction were shunted due to the neuron's ongoing hyperpolarization (compare Fig. 5.13A and B), indicating that these could not have been important for producing the neuron's discharge during those kicks which were triggered by nerve 5b1 stimulation. The few spikes elicited in the M-neuron at this time caused only momentary breaks in the discharge of contralateral flexors (see right leg EMG), but did not

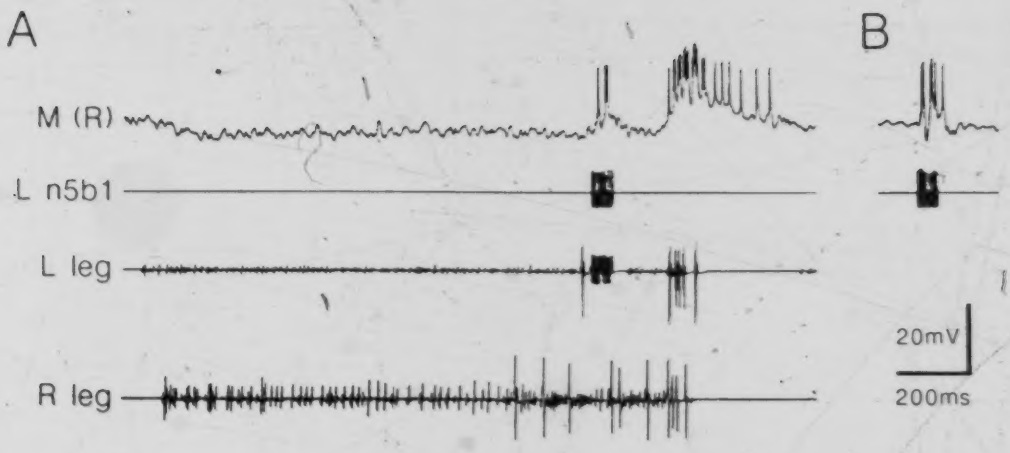

Fig. 5.13. Chordotonal stimulation does not evoke trigger activity or strongly activate the M-neurons when co-contraction has not been initiated. Top traces - intracellular recordings from the M-neuron which inhibits right leg flexors; second traces - 1.5 V, 200 Hz stimulation of the left nerve 5b1; bottom traces in A - left and right leg EMGs. A When CO afferents were stimulated in a leg in which the flexor and extensor muscles were not co-contracting, the motor program in the contralateral leg was not terminated after the usual delay. The pulse train evoked only weak synaptic input to the M-neuron, corresponding to a shunted version of the direct inputs evoked in the quiescent animal (B). Note that sensory stimulation only temporarily silenced ipsilateral flexor activity. Also, as is usual when co-contraction does not occur, there was a burst of FETi spikes in the left leg at the time of the terminal extensor burst in the right leg (cf. Fig. 6).

completely terminate activity in these motoneurons. The correlation noted between the inability of these pulse trains to evoke trigger activity and the absence of co-contraction in the stimulated leg suggests that the mechanisms underlying the generation of the co-contraction and trigger phases of the motor program may somehow be linked.

D. DISCUSSION

The major finding of this investigation is that kicking and jumping behaviors in the locust are dependent on tonic proprioceptive feedback from the hindleg CO. This receptor organ is essential for generating the co-contraction phase of the motor program and also appears to play a major, if not vital, role in producing the program's trigger phase. By contrast, the femoral lump receptor is not involved in the process by which kicks and jumps are triggered, and is of little, or no, importance in producing other aspects of the motor pattern. In this discussion, the evidence which supports these conclusions will be reviewed and the way in which these new findings influence current views concerning the neuronal mechanisms underlying the jump will be considered.

Lump Receptor Input Does Not Trigger Kicks and Jumps

One of the first indications that the lump receptor is not involved in the triggering process was that abolishing input from this receptor did not alter the ease with which kicks could be elicited. This contradicts the earlier finding of Heitler and Burrows (1977b) that it is more difficult to elicit kicks and jumps from a locust following ablation of the lump receptor. A possible explanation for this contradiction concerns the nature of the operation performed. It was noted during the course of these experiments that unless particular care was taken to minimize the severity of any dissection of the hindleg, an animal was often reluctant to kick after the operation. Thus, the conflicting results may reflect differences in the particular dissection procedures that were used.

When afferents in the lateral nerve, including those of the lump receptor, were electrically stimulated during co-contraction, premature extension of the ipsilateral leg was elicited due to the sudden termination of activity in the flexor motoneurons innervating that leg (Fig. 5.3). However, this shutdown of flexor activity was clearly not brought about by the natural triggering process. The M-neurons discharge strongly during the trigger phase of normal kicks (Chapters 2,3) and thus would be expected to do so whenever true trigger activity is elicited experimentally. Yet during kicks evoked by lateral nerve stimulation the M-neurons remained inactive (Fig. 5.4). These kicks could not, therefore, have been due to the activation of the central trigger system or to any direct excitation of the M-neurons themselves (cf. Pearson et al. 1980). Several other observations suggest that the triggering elicited by lateral nerve stimulation did not involve the trigger system. Firstly, because trigger activity in the two hindlegs during jumps and bilateral kicks is closely coupled, it might be expected that any sensory input that is able to evoke a kick from one leg should also exert some effect on the contralateral leg. Clearly, though, lateral nerve stimulation during co-contraction did not influence the motor program in the opposite leg because this sometimes ended several hundred milliseconds later (Fig. 5.4). Secondly, the typical pattern of motor activity during kicks includes a higher frequency burst of extensor spikes at about the time of trigger phase (Chapter 2). When leg extension was evoked prematurely by lateral nerve stimulation, FETi activity in the ipsilateral leg stopped suddenly and no terminal burst was evident (Figs. 5.3, 5.4). This suggested that the ongoing motor program in this leg was merely interrupted by the pulse train and, unlike the program in the unaffected contralateral leg, was not brought to its usual culmination. Finally, although lateral nerve stimulation terminated all spike activity in the ipsilateral flexors, it was never able to completely repolarize these motoneurons. There was always some subthreshold flexor excitation lingering until the normal time of trigger activity in the opposite leg (Fig. 5.3).

The probable cause of the kicks elicited by lateral nerve stimulation was a directly-evoked reflex inhibition of the ipsilateral flexor motoneurons. Support for this

conclusion comes from the finding that lateral nerve stimulation in the quiescent animal failed to activate the M-neurons and yet still evoked a strong hyperpolarization of the ipsilateral flexors (Fig. 5.5). Moreover, in each preparation the latency of this flexor hyperpolarization in the quiescent animal was identical to the latency of the repolarization produced prematurely in the motoneuron during co-contraction (Fig. 5.3). The "triggering" evoked by electrical stimulation of the lateral nerve, therefore, must simply have been caused by exciting the afferents responsible for the inhibitory reflex at a time when they would not normally have been active.

Based on these findings, it can be concluded that proprioceptive input from the lump receptor, or indeed from any of the afferents in the lateral nerve, does not contribute to the generation of the trigger phase of the kick or jump motor program. Moreover, the lump receptors do not appear to be important for producing the initial flexion or co-contraction phases of the program either, since abolishing lateral nerve input had no obvious effect on the pattern of motor activity during kicks (Fig. 5.1). They may still contribute in some way to the generation of flexor and extensor co-contraction, but if so, this role can only be a minor one. Considering that the lump receptors are activated only under the special circumstances in which the tibia is fully flexed and there is tension in the flexor tendon (Heitler and Burrows 1977b), i.e. the situation occurring before kicks and jumps, this apparent lack of importance is surprising.

Contribution of the Chordotonal Organ to the Motor Program

The Co-contraction Phase

Ever since the initial reports that locusts no longer jump after transection of the CO apodeme or nerve (Bässler 1969; Usherwood et al. 1968), it has generally been assumed that the role of this receptor organ is to generate the trigger phase of the motor program (e.g. Godden 1975; Pearson et al. 1980). The results of the present study, however, demonstrate that the reason locusts are unable to jump or kick after either of

these operations is because the co-contraction phase of the program does not occur. The operated legs were often fully flexed as normal, but simultaneous fast extensor activity was never initiated. Consequently, without co-contraction of the femoral flexor and extensor muscles, the energy which would normally power the ballistic extension of the hindlegs cannot be stored or subsequently released.

In one report which cast doubt on the importance of the CO, it was claimed that animals whose CO apodemes have been cut regain their ability to jump several days after the operation (Bässler 1979). However, EMG analyses in the present study showed that this is not true. The behavior of these animals outwardly resembled jumping, but leg extension was produced only by brief bursts of fast extensor activity, and was not preceded by co-contraction of the flexor and extensor muscles. Presumably, the range of these "jumps", and the acceleration achieved during take-off, would be considerably reduced in comparison with jumps in an intact animal.

Details of the way in which feedback from the chordotonal organ contributes to the production of flexor and extensor muscle co-contraction are not known. Heitler and Bräunig (1988) recently demonstrated that neither the central connections of the FETi motoneurons nor the peripheral feedback due to extensor muscle tension are important for generating the pattern of activity in flexors and extensors during the co-contraction phase. This, together with the finding that co-contraction is abolished by cutting the CO receptor apodeme, an operation which eliminates the organ's responses in the 0-80° range of femoral-tibial angles (Zill 1985), suggests that the afferent signals that are critical for the generation of co-contraction are those encoding some aspect of leg flexion. The CO has no direct mechanical linkages to flexor muscle fibres and consequently does not respond to contractions of the flexor muscle (Zill 1985). Thus, the organ cannot signal flexor tension during the motor program. The essential feedback from the CO must, therefore, be tonic afferent activity encoding the flexed position of the tibia. Considering that tonic units in the CO respond only in defined (and sometimes narrow) portions of

the range of femoral-tibial joint angles (Zill 1985), a very small number of receptors may provide the joint angle information that is necessary for the generation of co-contraction. The mechanisms by which this proprioceptive feedback is integrated centrally during the motor program remain to be determined.

The Trigger Phase

It was not possible in these experiments to determine unequivocally that the CO is necessary for the generation of the trigger phase because eliminating input from this receptor also prevents the preceding co-contraction phase from occurring. However, proprioceptive feedback from the CO does appear to play a role in producing trigger activity. In support of this conclusion is the finding that chordotonal stimulation during co-contraction consistently led to the termination of motor activity in the contralateral leg, regardless of whether the stimulus was delivered early or late in the co-contraction phase (see EMG traces in Figs. 5.8, 5.10-12). Consequently, a clear causal relationship exists between the time of stimulation during the motor program and the duration of co-contraction in the opposite leg (Fig. 5.9). As mentioned previously, trigger activity on the two sides is normally closely coupled and so this sort of contralateral sensory influence is precisely what would be expected if the natural triggering process were being invoked. Furthermore, stimulation of the CO afferents during co-contraction always powerfully activated both M-neurons (Figs. 5.11, 5.12) and caused the steep repolarization of contralateral flexor motoneurons (Fig. 5.10), all with similar latencies. The depolarizations evoked in the M-neurons usually closely resembled those ocurring during normal kicks, except that there were extra initial components produced as a direct result of the chordotonal afferent stimulation. Since the same stimulation in the quiescent animal had little effect on the M-neurons (Figs. 5.11C, 5.12C) and no effect on the contralateral flexors (Fig. 5.10C), the responses elicited during co-contraction must have been produced by afferent excitation of the central trigger system. This is also indicated by the observation that if a pulse train fails to terminate co-contraction in the opposite

leg, it also does not produce a powerful discharge in the M-neurons (Fig. 5.13). The trigger discharge in the M-neurons, therefore, cannot simply be a direct response to the proprioceptive input and must instead result from the excitation of other interneurons. These findings provide further support for the existence of a central trigger system. Yet more evidence indicating the involvement of the CO in the natural triggering process is that when stimulus trains during co-contraction produced trigger activity in the contralateral leg, the EMG pattern in this leg always concluded with a high frequency burst of FETi spikes. This is a usual feature of the motor pattern of bilateral kicks (Chapter 2) and, therefore, indicates that chordotonal stimulation led to the normal termination of the program.

In addition to its actions via the central trigger system, the CO exerts influences on ipsilateral flexor motoneurons by more direct pathways (see also Burrows 1987). These pathways were activated whenever the CO afferents in nerve 5b1 were electrically stimulated. Thus, the reflex inhibition of ipsilateral flexors evoked in the quiescent animal (Fig. 5.8C) was also evoked by stimulation during co-contraction, where it caused the sudden termination of all spike activity in these flexors at least 20 ms before the onset of the discharge in the M-neurons and the trigger-associated repolarization of contralateral flexors (compare Fig. 5.8A,B with Figs. 5.10-12). The immediate effect of chordotonal stimulation was, therefore, similar to that produced by lateral nerve stimulation in that it simply interrupted motor activity in the ipsilateral leg. This was also indicated by the absence of the usual extensor burst at the time of the pulse train (Fig. 5.8). However, unlike what was observed for lateral nerve stimulation, shortly after the pulse train was applied to nerve 5b1, true trigger activity was evoked and this ensured the complete repolarization of the ipsilateral flexor motoneurons.

The conclusion that feedback from the CO is able to produce trigger activity is based on the results of electrical stimulation of nerve 5b1. However, this nerve contains sensory axons in addition to those from the CO and these other afferents would also have

been activated by the pulse trains delivered during co-contraction. Because destroying the tibial sense organs which have axons in nerve 5b1 (i.e. the subgenual organ, Heitler and Burrows 1977b) had no major effect on the motor program, it appears unlikely that sensory input from these receptors would be important for generating the trigger signal. Other sensory axons that have been reported to run in nerve 5b1 are those of tactile hairs, but their potential role in the triggering process can almost certainly be discounted. This, together with the results of specific ablation experiments indicating that chordotonal input is vital for the production of the motor program, makes it seem reasonable to attribute the observed trigger-associated effects of nerve 5b1 stimulation on interneurons and motoneurons to the chordotonal organ.

Given that the CO is involved in producing trigger activity, an obvious question is what is the nature of the proprioceptive information it provides during this process? Heitler and Bräunig's (1988) study indicated that sensory feedback related to extensor motor output is not only unimportant for generating the co-contraction phase of the program, but also that it is not necessary for the trigger phase to occur. Thus the CO's responses to resisted extensor muscle contractions (Fig. 5.7B; Zill 1985) are probably not essential for the production of trigger activity. Since recordings of chordotonal afferent responses during kicks show no obvious sign of a phasic discharge prior to the rapid repolarization of flexor motoneurons (Fig. 5.7), tonic feedback from the CO signalling the leg's full flexion may be responsible for producing trigger activity, just as it appeared to be critical for generating co-contraction. The question now arises of how this sensory feedback during the motor program can lead to the generation of the trigger phase. One possible mechanism is that the tonic afferent input to the CNS during co-contraction is integrated in such a way as to gradually bring the trigger system to its activation threshold. Presumably, electrical stimulation of the CO afferents simply accelerates this process. Consistent with this idea was the finding that pulse trains during co-contraction did not evoke trigger activity immediately, but rather after a variable delay of some 40-60

ms. It is curious that the length of this delay was not dependent on the time of stimulation during co-contraction (Fig. 5.9) for it might have been expected that a pulse train early in co-contraction would take longer to activate the trigger system than one delivered close to the normal time of trigger activity. Another possible mechanism is that the co-contraction and trigger phases are intimately linked, such that chordotonal feedback is involved in generating co-contraction and the interneurons which produce this phase of the program are responsible for activating the trigger system. Either one of these mechanisms would account for the observation that trigger activity cannot be evoked by stimulating CO afferents in a leg in which co-contraction has not been initiated (Fig. 5.13). Apparently, the particular excitatory inputs which are necessary for the initiation and maintenance of co-contraction are also required for triggering to occur.

Concluding Remarks

Bässler (1968) concluded that a locust can only jump when complete flexion of the femoral-tibial joint has been signalled by the CO. The present findings uphold this original proposal, although they do indicate that the CO's involvement in the behavior is not quite so simplistic. The CO has been shown to be critical for the generation of the co-contraction phase, but it was not possible to demonstrate unequivocally whether this was also true for the trigger phase. However, since the M-neuron depolarizations produced by chordotonal stimulation were similar in both form and strength to those which occur during normal kicks, it is tempting to speculate that feedback from the CO may be the only sensory input necessary to produce trigger activity. Consistent with this idea is that no other hindleg proprioceptor has been found to be important for the triggering process, and also, as was shown in the preceding chapter, exteroceptive inputs cannot trigger kicks and jumps. The appeal of this suggestion is that regardless of the context in which kicking and jumping behaviors are performed (i.e. escape, self-defense or locomotion), hindleg extension could be triggered by the same sensory signals.

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VI. COUPLING OF FLIGHT INITIATION TO THE JUMP[3]

A. INTRODUCTION

It has been known since the early work of Weis-Fogh (1949, 1956) that the production of sustained flight in locusts requires a maintained stream of air on the head and the loss of tarsal contact with the ground. Since flight activity in adult animals is normally initiated following a jump, it is probable that both the self-generated stream of air on the head produced by the jump and the associated loss of tarsal contact with the ground play a role in the initiation of flight activity. However, these are not the only two factors involved in flight initiation. In a series of behavioral studies Camhi (1969) showed that the onset of wing opening often began too soon after triggering of the jump for either wind on the head or the loss of tarsal contact to be responsible for initiating flight activity. Subsequently, Pond (1972b) reported that covering the head-hair receptors or cutting the neck connectives did not prevent flight initiation following a jump. In fact, both these procedures reduced the mean delay between triggering of the jump and the start of wing opening, and in some trials wing opening commenced before the loss of tarsal contact with the ground. Although the exact timing of the onset of flight activity relative to the triggering of the jump has not been established using EMG techniques, it can be estimated from the observations of Camhi and Pond. Camhi (1969) found that the wing opening often began less than 15 ms following the onset of a jumping movement and Pond (1972a) established that activity in elevator motoneurons commenced 10 to 40 ms before the first visible wing movements. From these observations it follows that the onset of activity in elevator motoneurons must commence close to, and sometimes before, the time the jump is triggered. The simplest hypothesis to explain these observations is that the system responsible for programming the motor sequence

[3]A version of this chapter has been published. Pearson KG, Gynther IC, Heitler WJ (1986) J Comp Physiol 158:81-89

for the jump also activates the flight motor system, possibly via a central pathway in the nervous system (Camhi 1969). Consistent with this hypothesis is that a group of interneurons (designated 404 neurons) capable of initiating flight activity receive a strong excitatory input close to the time hindleg kicks are triggered (Pearson et al. 1985). This observation suggested a more specific hypothesis: namely that it is the system which causes the jump to be triggered which initiates activity in the flight system.

If this hypothesis were true, there should be an invariant temporal relationship between the time hindleg kicks are triggered and the onset of flight activity. In particular, the flight elevator motoneurons would be expected to receive significant synaptic input close to the time kicks are triggered. The aim of this investigation was to test these predictions by recording the timing of spike activity and the pattern of synaptic activity in identified flight motoneurons and interneurons during bilateral kicks of the hindlegs.

B. MATERIALS AND METHODS

Experiments were performed on male and female *Locusta migratoria* obtained from a colony at the University of Alberta. The preparation used in this investigation was identical to that described in the preceding chapters except that additional EMG recording electrodes were placed in the dorsal longitudinal muscles to monitor flight activity. Intracellular recordings were made from identified flight motoneurons and interneurons using the techniques described by Robertson and Pearson (1982). Although it was possible to identify motoneurons and many interneurons using physiological criteria (Hedwig and Pearson 1984; Robertson and Pearson 1983, 1985), the neuron from which recordings were made was routinely stained with the fluorescent dye Lucifer Yellow. Knowledge of the structure and physiological properties allowed the identification of interneurons and the main flight motoneurons in the meso- and metathoracic ganglia (occasionally it was not possible to distinguish between the anterior tergocoxal and tergotrochanteral motoneurons). Recording electrodes were filled with a 5% solution of Lucifer Yellow in distilled water. Dye was injected by passing a constant -5 nA

current for 5 min. Ganglia were fixed in 4% paraformaldehyde, dehydrated, cleared in methyl salicylate, and viewed as wholemounts.

C. RESULTS

EMG Recordings From Flight and Leg Muscles

An early observation in this study was that a short sequence of flight activity was often evoked when both hindlegs kicked. The initial aim, therefore, was to establish the timing of the onset of this flight activity relative to the time kicks were triggered. To do this, EMGs were recorded from the extensor and flexor tibiae muscles of both hindlegs and from the dorsal longitudinal muscles, and kicks were elicited by light mechanical stimulation of the abdomen. In the restrained preparation used in this investigation, kicks were produced almost simultaneously in both hindlegs (Fig. 6.1), and the pattern of motor activity in metathoracic extensor and flexor tibiae muscles was similar to that for a jump (Chapter 2). This pattern consisted of a brief period of flexor activity followed by a short period (about 400 ms) of co-contraction of both sets of muscles. The co-contraction phase was terminated by a sudden cessation of flexor activity, and extensor activity then ceased about 20 ms later. Because the large spike from the single fast motoneuron (FETi) to the extensor muscle was very obvious in the leg EMG recordings (Fig. 6.1), it was decided to take the last extensor spike as the reference point for measuring the timing of the onset of flight activity. The rhythmic discharge of dorsal longitudinal motoneurons is referred to as flight activity although this pattern is clearly different from that in intact animals. In restrained dissected preparations the rhythmic activity recorded from dorsal longitudinal muscles occurs at about 10/s (Figs. 6.1, 6.2), which is about half the normal flight frequency.

The EMG recordings from the flight and leg muscles showed that flight activity commenced at a variable time relative to the time a kick was triggered. Figure 6.1 shows the depressor activity beginning close to the time the kicks were triggered, while Fig. 6.2 shows

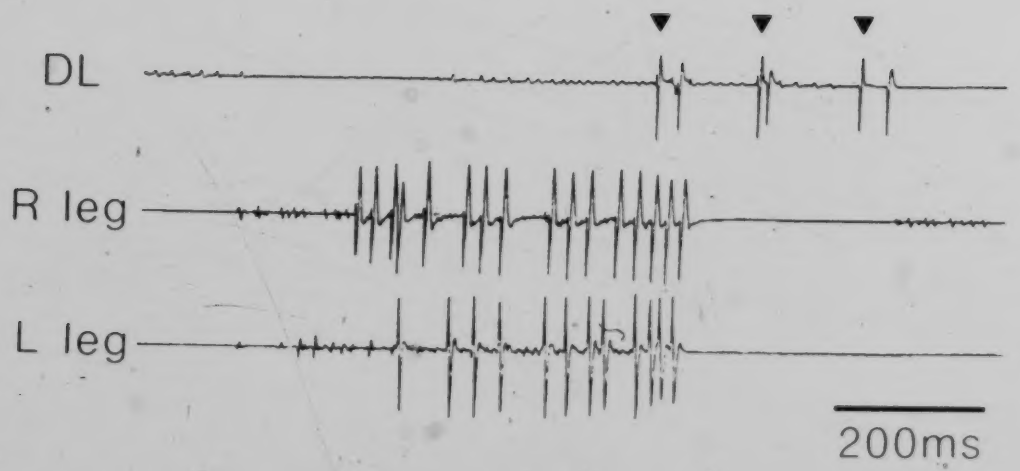

Fig. 6.1. Kicking in both hindlegs of a restrained locust evokes a short sequence of flight activity. Top trace (DL) - EMG recording from the dorsal longitudinal (depressor) muscle; bottom two traces - EMGs from the flexor and extensor tibiae muscles of the two hindlegs. In this example a short sequence (3 cycles) of rhythmic activity in the dorsal longitudinal muscle (large spikes in top record) commenced just before the triggering of the kicks. Two spikes occurred for each cycle. Arrowheads indicate the first spike in each doublet.

depressor activity beginning mid-way through the co-contraction phase. The extent of this variation in the interval between the first spike in the dorsal longitudinal motoneurons and the last spike in the ipsilateral FETi is illustrated in the histogram in Fig. 6.3. This histogram summarizes data obtained from 58 kicks in 12 animals with a minimum of three kicks occurring in each animal. For almost one-half (48%) of the kicks, the dorsal longitudinal activity commenced during the co-contraction phase of the kick, i.e. during the time FETi was active (see examples in Figs. 6.1, 6.2). This fact, together with the finding that the onset of flight activity was not always synchronous with the time kicks were triggered, indicates that the trigger system alone is not responsible for initiating flight activity. A possible explanation for the variability in the timing of flight onset relative to the time kicks were triggered is that both events were initiated independently by the abdominal stimulus. Although abdominal stimulation can lead to flight activity in the absence of kicking, there are three reasons for believing that the flight activity associated with kicks was not the direct result of abdominal stimulation. Firstly, bilateral kicks sometimes occurred many seconds after abdominal stimulation had ceased but flight activity was still initiated during the co-contraction phase of these spontaneous kicks. Secondly, there was no strong temporal relationship between the time of abdominal stimulation and the onset of co-contraction. Typically this interval varied from zero to two seconds yet the onset of flight activity was almost always within 100 ms of the time kicks were triggered. Thirdly, occasionally a single abdominal stimulus led to a series of kicks (2 or 3) and an excitatory influence was observed on the flight system during the co-contraction phase of all kicks in the series. This excitatory effect was usually manifested as a progressive decrease in the cycle-period (i.e. increase in frequency) of the flight rhythm during the co-contraction phases (see Fig. 6.2 for a single example).

The fact that the probability of initiating flight activity increased progressively near the end of the co-contraction phase (Fig. 6.3), together with the observation that the cycle-period of the flight activity decreased progressively during co-contraction (Fig. 6.2), suggests that during co-contraction there is an increasing excitatory input to the flight system.

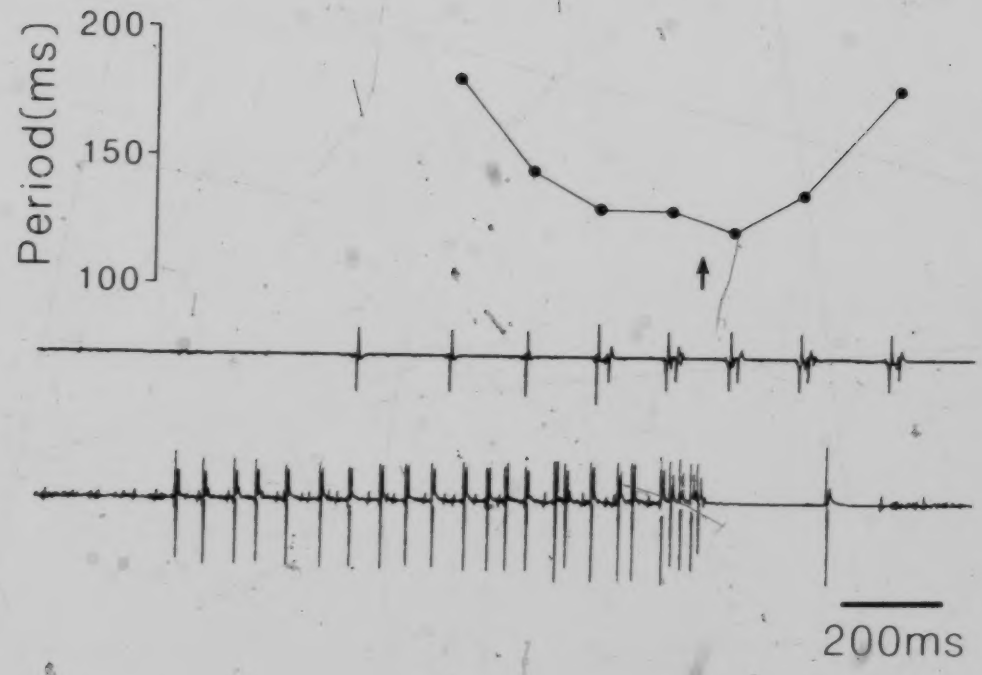

Fig. 6.2. Progressive increase in the excitation of the flight system during the co-contraction phase of a kick. Bottom trace - EMG from the right hindleg muscles showing the motor activity associated with the co-contraction phase of the kick; top trace - EMG from the right dorsal longitudinal muscle. Note that a rhythmic sequence of activity in dorsal longitudinal motoneurons commenced early in the co-contraction phase and the frequency and intensity of flight activity increased throughout the remainder of the co-contraction phase. The graph at the top plots the cycle period of the dorsal longitudinal activity. Each point represents the immediately preceding interval. Note that the shortest cycle interval occurred at the time leg EMG activity ceased, i.e. close to the moment the kick was triggered (arrow).

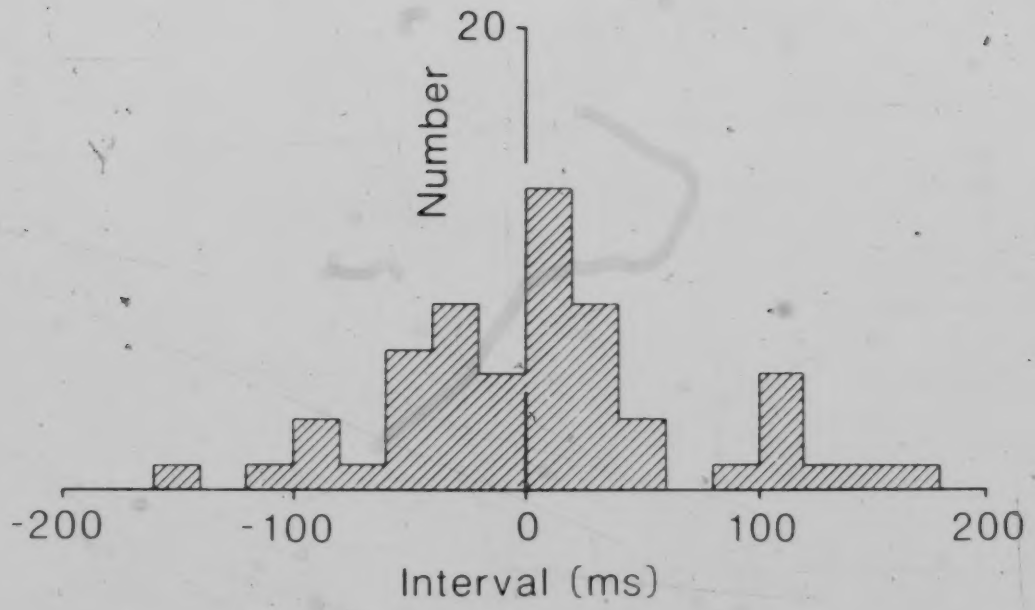

Fig. 6.3. Histogram showing the distribution of the interval between the approximate time a kick was triggered (measured as the last extensor spike in the co-contraction phase) and the time of occurrence of the first spike in the EMG from the dorsal longitudinal muscles. Negative values of this interval mean that the first dorsal longitudinal spike occurred before the last fast extensor spike. This histogram was constructed from observations on 12 animals, all of which gave three or more kicks.

In addition, the EMG recordings do not exclude the possibility that the flight system also received a transient excitatory input at the time kicks were triggered. This would be consistent with the finding that the probability of initiating flight activity was maximal immediately following the termination of activity in FETi (Fig. 6.3).

Intracellular Recordings From Flight Motoneurons and Interneurons

In order to gain more insight into the mechanism for the coupling of flight activity with kicking, intracellular recordings were made from a variety of flight motoneurons and interneurons during bilateral kicks of the hindlegs. Based on the observations from EMG recordings, it was of interest to determine which flight neurons were excited during the co-contraction phase and whether any flight neurons received a phasic input specifically associated with the triggering of the kicks.

Recordings were made, during kicks, from flight motoneurons innervating all the main flight muscles in the meso- and metathoracic ganglia (see Hedwig and Pearson 1984, for a description of the physiology and anatomy of flight motoneurons). No attempt was made to record from the motoneurons innervating the pleuroalar muscle which is used primarily for controlling wing twisting (Heukamp 1984). To date, no consistent differences have been noted in the pattern of synaptic activity in the different members of each of the two sets of flight motoneurons (elevators and depressors) during kicks. Thus, in the descriptions that follow, the different types of elevator motoneurons or the different types of depressor motoneurons have not been distinguished between, although the identity of the various motoneurons is noted in the figure legends.

Since flight activity evoked by wind on the head or the loss of tarsal contact with the ground begins with excitation of elevator motoneurons (Pond 1972b), the input to these motoneurons during kicks was examined first. Flight sequences associated with hindleg kicks always began with a depolarization and burst of activity in elevator motoneurons (Fig. 6.4). The first depolarization leading to spikes in elevator motoneurons began from 50 to 200 ms

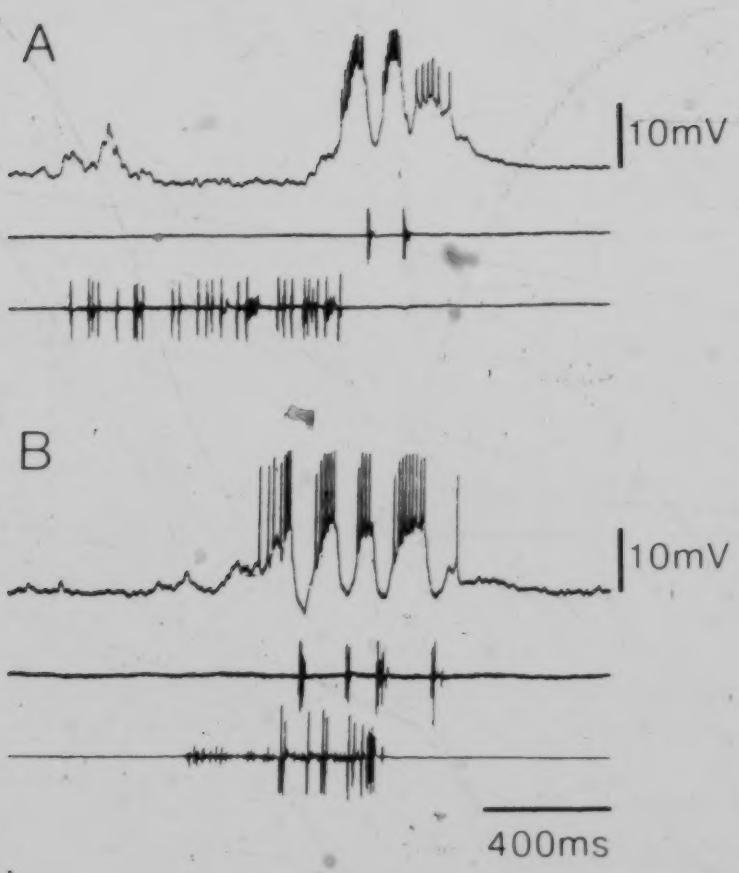

Fig. 6.4. Intracellular recordings from wing elevator motoneurons during kicks of the hindlegs. Top traces - intracellular recordings; middle traces - EMGs from dorsal longitudinal muscle; bottom traces - EMGs from extensor and flexor tibiae muscles. A Mesothoracic anterior tergocoxal motoneuron. The onset of rhythmic oscillations in this example occurred close to the time a kick was triggered (indicated approximately by the sudden termination of activity in the leg EMG). B Mesothoracic posterior tergocoxal motoneuron. In this sequence the motoneuron became active before onset of co-contraction. Note in B that the termination of the co-contraction phase occurred at the time the motoneuron was becoming hyperpolarized whereas in A it occurred when the motoneuron was depolarized. Note also that the initial spike activity in the elevator motoneurons preceded the first spikes in the dorsal longitudinal muscle.

before the first spike in the dorsal longitudinal EMG. The number of recordings made from elevator motoneurons was not sufficient to construct an accurate distribution of the time the elevators were excited during kicks, but they were sufficient to show a high degree of variability in this timing. For example, an elevator motoneuron was sometimes observed to be excited close to the time a kick was triggered (Fig. 6.4A) while at other times the initial depolarization commenced during the flexor activity which preceded the co-contraction phase (Fig. 6.4B). This variability in the time of onset of depolarization in elevator motoneurons was consistent with the variability seen in the occurrence of the first dorsal longitudinal spike seen in EMG recordings. The dorsal longitudinal spikes occurred soon after the first burst of activity in the elevator motoneurons (Fig. 6.4).

A major interest was to establish whether or not the elevator motoneurons received a phasic excitatory input at the time the kicks were triggered. This required some knowledge of how kicks are triggered. Intracellular recordings from interneurons involved in triggering the kick (the M-neurons, Pearson et al. 1980) have shown that the beginning of the high frequency discharge in these neurons occurs 23 ± 16 ms before the last extensor spike (Chapter 2). Thus, if elevator motoneurons (or any other neurons) receive input from the trigger system, the timing of this input would be expected to occur near the end of extensor activity. Although some recordings did show a strong input closely timed to triggering (Fig. 6.4A) this was not common. More often there was no obvious additional input at the time of triggering. For example, the time of triggering of the kick in Fig. 6.4B was associated with a hyperpolarization (cf. a depolarization in Fig. 6.4A) and there was no obvious input which disrupted the rhythm. A general observation was that when rhythmic activity was elicited prior to the triggering of a kick the rhythm was not disrupted at the time of triggering *regardless* of the phase of the oscillation at the moment of triggering.

Another indication that the elevator motoneurons do not receive a phasic excitatory input at the time of triggering of a kick was that these motoneurons were often tonically depolarized during the co-contraction phase and there was no sign of additional synaptic input

at the time of triggering (Fig. 6.5). Not all recordings from elevators showed this tonic excitation during co-contraction. For example, the recording in Fig. 6.4A shows that the motoneuron was slightly hyperpolarized during most of co-contraction. Hyperpolarizing responses were rare in elevator motoneurons and their occurrence has not been correlated with any particular type of elevator motoneuron.

Intracellular recordings from depressor motoneurons (Fig. 6.6) also failed to reveal a significant synaptic input time-locked to the triggering of the kick (Fig. 6.6A). Depressor motoneurons were usually weakly hyperpolarized during co-contraction (cf. depolarization in elevators, Fig. 6.5) and this hyperpolarization was terminated either by the beginning of rhythmic oscillations (Fig. 6.6B) or by a return of the membrane potential to the pre-kick level (Fig. 6.6A). As with the elevators, rhythmic oscillations in the membrane potential were not disrupted at the time of triggering.

In summary, neither elevator nor depressor motoneurons received a phasic synaptic input at the time kicks were triggered and, with few exceptions, elevator and depressor motoneurons show different responses during the co-contraction phase : elevators often being tonically depolarized and depressors being weakly hyperpolarized. The latter responses are somewhat similar to the pattern of synaptic input to flight motoneurons produced by a wind stimulus to the head, all elevators being depolarized by wind and many depressors being hyperpolarized (Hedwig and Pearson 1984). These observations, therefore, suggest that common mechanisms are involved in activation of the flight system by co-contraction and by wind on the head. If this conclusion were correct, then the pattern of synaptic input to flight interneurons during co-contraction should be similar to the pattern in response to wind on the head. This indeed was the case, for in the interneurons examined so far there was a good correlation between the initial response to wind and the polarity of the synaptic input during co-contraction (Table 6.1, Fig. 6.7).

Another aim of the study of the responses of flight interneurons during kicking was to identify those interneurons that might be involved in mediating the coupling between the

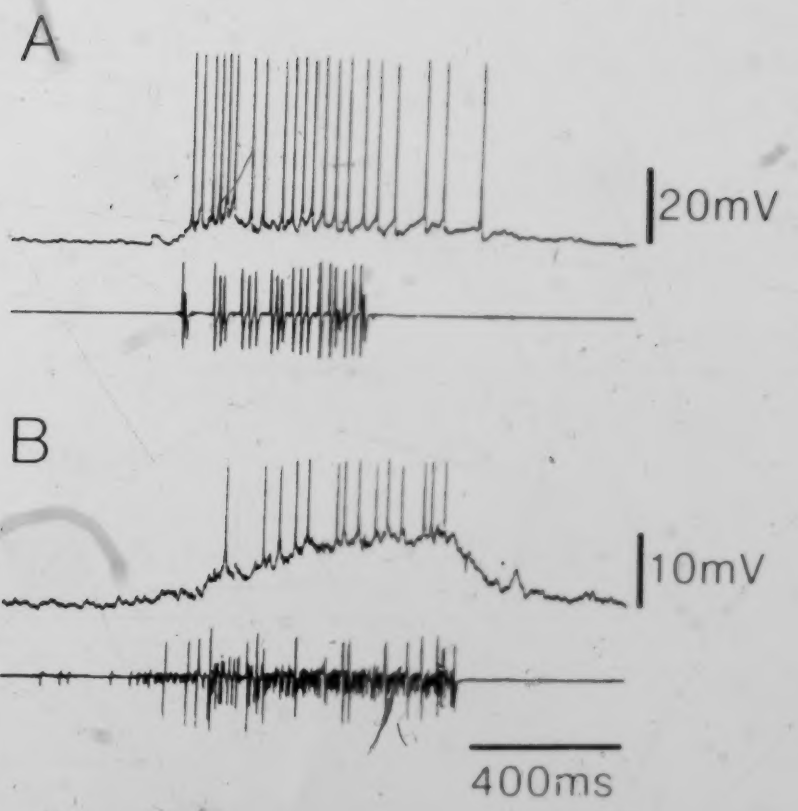

Fig. 6.5. Intracellular recordings from elevator motoneurons during kicks when rhythmic flight activity was not initiated. Top traces - intracellular recordings; bottom traces - EMGs from flexor and extensor tibiae muscles of the right hindleg. **A** Metathoracic tergosternal motoneuron. **B** Mesothoracic anterior tergocoxal motoneuron. Note the tonic excitatory input to the motoneurons during the co-contraction phase and the absence of any additional input at the approximate time of triggering, i.e. at the cessation of the leg EMG activity.

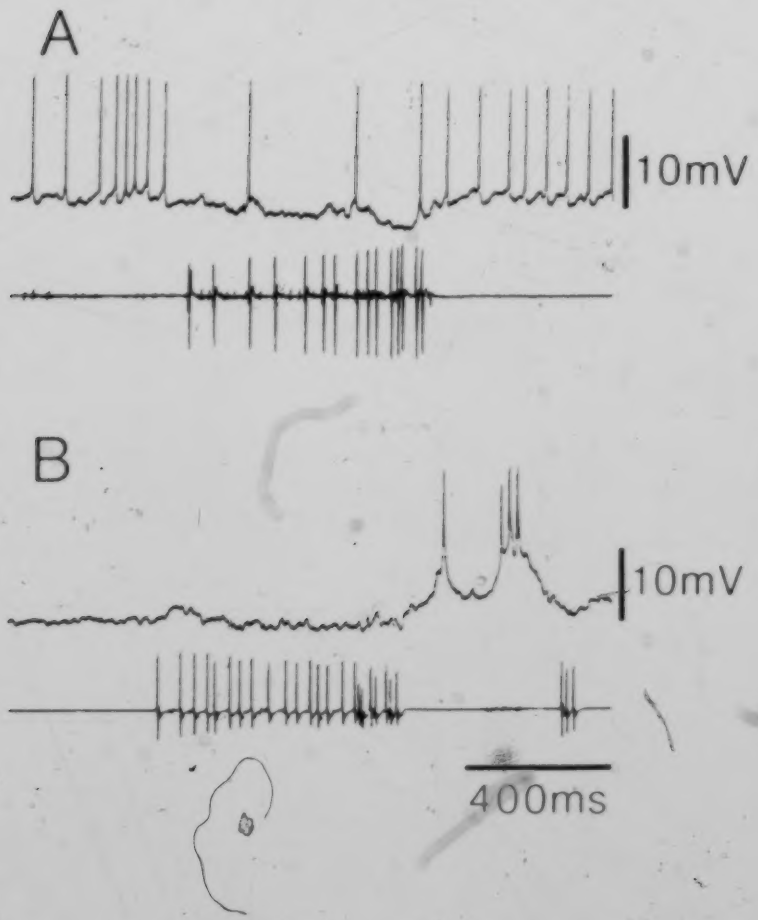

Fig. 6.6. Intracellular recordings from depressor motoneurons during kicks. A Mesothoracic subalar motoneuron. Rhythmic flight activity was not evoked during this kick. Note hyperpolarization of the motoneuron (top traces) during the co-contraction phase and absence of any phasic input at the time the kick was triggered. Bottom trace - EMG from the left hindleg extensor and flexor tibiae muscles. B Mesothoracic subalar motoneuron in another preparation. The intracellular record (top trace) shows the motoneuron to be slightly hyperpolarized during the co-contraction phase indicated by the activity in the hindleg flexor and extensor tibiae muscles (bottom trace). Oscillations in the membrane potential of the motoneuron commenced after the kick was triggered.

Table 6.1.

Neuron	Initial response to wind	Response during co-contraction	No. of observations
201	hyperpolarization	hyperpolarization	1
301	hyperpolarization	hyperpolarization	3
302	depolarization	depolarization	1
401	depolarization	depolarization	6
501	hyperpolarization	hyperpolarization	4
504	depolarization	depolarization	5
515	depolarization	depolarization	3

Table 6.1. Correlation between the initial response to wind on the head and the response during co-contraction in identified flight interneurons. The structure and physiology of these neurons has been described by Pearson and Robertson (1983).

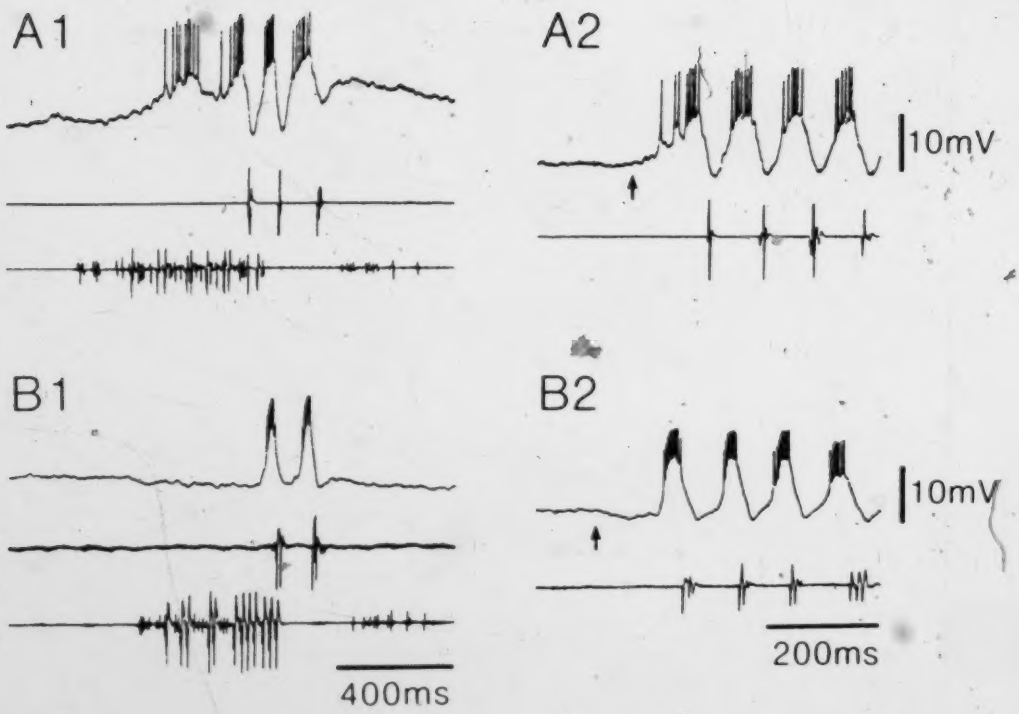

Fig. 6.7. Intracellular recordings from identified flight interneurons during a kick (A1 and B1) and when wind is blown on the head (A2 and B2). Top traces - intracellular records; middle traces in A1 and B1 and bottom traces in A2 and B2 - EMG recordings from dorsal longitudinal muscles; bottom traces in A1 and B1 - EMG from left hindleg extensor and flexor tibiae muscles. Interneuron 401 (A1 and A2) was depolarized during the co-contraction phase of the kick and showed a few cycles of rhythmic activity beginning slightly before the triggering of the kick. 401's initial response to the wind was also a depolarization (onset indicated by arrow). Interneuron 301 (B1 and B2) was hyperpolarized during the co-contraction phase of the kick (B1) and initially hyperpolarized (onset indicated by arrow) by wind on the head (B2). Physiology and anatomy of the 301 and 401 neurons have been described elsewhere by Robertson and Pearson (1983).

kicking and flight systems. This investigation sampled only a fraction of the interneurons which have so far been identified (the number is now over 60). Nevertheless, recordings were made from two interneurons which were particularly strongly excited during the co-contraction phase (Fig. 6.8). These were the 401 and 504 neurons in the metathoracic ganglion (see Robertson and Pearson 1983, for a description of their structure). The fact that these two interneurons are excited during co-contraction is of interest for three reasons: (1) both are members of the central flight oscillator, (2) both are strongly excited by wind on the head, and (3) their known connections are consistent with the synaptic input observed in flight motoneurons during co-contraction. Interneuron 504 excites elevator motoneurons and interneuron 401 inhibits depressor motoneurons. These data suggest that the 401 and 504 neurons are involved in linking the kicking system to the flight system. However, more extensive information on the interconnections between these and other interneurons is clearly needed before any firm statements about the pathways linking the kicking and flight systems can be made.

D. DISCUSSION

In this investigation it has been demonstrated that evoking the motor program for kicking of the hindlegs can elicit flight activity in restrained locusts. This supports an earlier suggestion that flight activity in normal adult animals can sometimes be initiated via pathways linking the jumping system to the flight system (Camhi 1969). This study was begun with the idea that it was the triggering of the kicks which led to the activation of the flight system. This idea arose from the finding that a set of identified interneurons capable of initiating flight activity when depolarized were phasically excited at the time kicks were triggered (Pearson et al. 1985). None of the present data supports this idea, however. Firstly, elevator motoneurons, which are the first motoneurons to become active at the beginning of flight, were not found to be consistently depolarized close to the time kicks were triggered (Fig. 6.4). Secondly, there was no disruption of rhythmic activity in flight motoneurons and interneurons

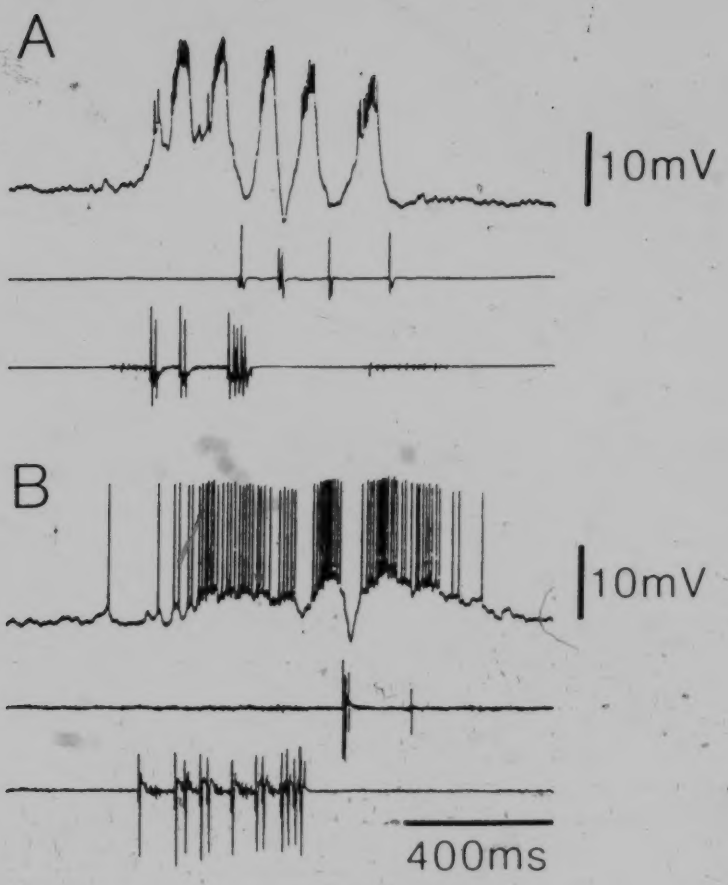

Fig. 6.8. Intracellular recordings from two flight interneurons showing a strong depolarizing response during the co-contraction phase of a kick. **A** Interneuron 504. **B** Interneuron 401 (not the same neuron as in Fig. 6.7A). Top traces - intracellular recordings; middle traces - EMGs from right dorsal longitudinal muscles; bottom traces EMGs from right hindleg extensor and flexor tibiae muscles. Both interneurons received a strong depolarization during co-contraction and interneuron 504 generated two bursts of spike activity on the plateau of the depolarization. The initial response of both interneurons to wind on the head was also a depolarization. Physiology and anatomy of the 504 and 401 interneurons have been described elsewhere (Robertson and Pearson 1983).

at the time of triggering (triggering could occur with the flight neurons in either a hyperpolarized or a depolarized phase). Finally, in some preparations in which flight activity was not evoked there was no significant synaptic input to flight motoneurons at the time of triggering (Figs. 6.5, 6.6A). Although all these data indicate a lack of coupling of the triggering system to the flight system, there is the possibility that a pathway linking these two systems is non-functional in the preparation used in this study. This seems unlikely, however, because no sign of linkage was seen in animals displaying a wide range of excitability in their flight systems, from fully depressed to highly active.

What was clear in the recordings was that flight activity often commenced during the co-contraction phase of the kick (Figs. 6.2, 6.3), and that flight elevator motoneurons and interneurons usually received a substantial input during co-contraction (Figs. 6.4, 6.5, 6.8). The simplest explanation for these observations is that co-contraction produces an excitatory input to the flight system to initiate flight activity (flight always begins with the excitation of elevator motoneurons). Another explanation is that the abdominal stimulation used to evoke kicks may have directly excited the flight system and that co-contraction had no influence on the flight system. This explanation is considered to be unlikely, however, because of the variable temporal relationship between abdominal stimulation and the occurrence of kicks. On some occasions, kicks occurred spontaneously many seconds following the abdominal stimulus yet the initiation of flight activity was still associated with the co-contraction phase of the kick. Another indication that co-contraction leads to excitation of the flight system was the progressive decrease in the cycle-period when flight activity was initiated early in co-contraction (Fig. 6.2). This augmenting input to the flight system was also seen in some intracellular recordings from flight motoneurons (Fig. 6.5B) and interneurons (Fig. 6.7A1). Given that the flight system does receive excitatory input during co-contraction, two obvious questions arise: what is the origin of the excitatory input, and what prevents the initiation of flight activity during co-contraction in unrestrained intact animals?

Unfortunately, very little can be said about the origin of the excitatory input to the flight system during co-contraction. Currently, the mechanisms by which the co-contraction phase is initiated and maintained are only partially understood. It is clear that one important factor involved in keeping extensor and flexor tibiae motoneurons active during co-contraction is sensory feedback from the hindleg chordotonal organ (Chapter 5). However, very little is known about the pathways from the chordotonal afferents to leg motoneurons. Certain spiking local interneurons have been found to be important for processing information in reflex pathways from the chordotonal organ (Burrows 1987), but the interneurons which are likely to mediate sensory influences on flexors and extensors during co-contraction have not been identified. It is known, though, that interneurons not in the flight system are strongly activated during the co-contraction phase (Chapter 2). Whether some of these provide the excitatory input to the flight system, as well as playing some role in maintaining the co-contraction phase, has not yet been established.

The question of why flight activity is not initiated during co-contraction in intact animals may have a simple explanation. Normally in intact animals there is a strong inhibitory input from tarsal receptors to the flight system. This input was not present in the preparation used in this investigation (the hindlegs were transected and the fore- and middle legs were removed) so any excitatory input to the flight system would be more likely to initiate rhythmic activity in this system. Presumably in the intact animal the tarsal inhibition is of sufficient magnitude to ensure that flight activity is prevented until near the end of the co-contraction phase. Because this balance between excitatory and inhibitory input depends on factors such as the intensity of co-contraction and the strength of tarsal contact with the ground, and because there is no indication of significant input from the trigger system, considerable variation would be expected in the time of onset of flight activity relative to the time a jump is triggered. This was observed by Pond (1972a) in EMG recordings from flight muscles during jumps. It should also be noted that intact animals do not always fly following a jump. Thus it must be concluded that input during co-contraction is not always sufficient

to initiate flight activity. There is evidence that the head ganglia exert a regulatory influence on the flight system (for example, Pond (1972b) showed that cutting the neck connectives reduced the time of wing opening following a jump). Thus, whether or not a jump leads to flight activity presumably depends to some extent on the level of central modulatory influences on the flight system.

Figure 6.9 summarizes the conclusions about how the flight system is linked to the jumping system in the intact animal and some of the factors controlling the initiation and maintenance of flight activity. Not included in this schematic are central inputs regulating the excitability of the flight system, and inputs from wind-sensitive afferents from the wings and body (Weis-Fogh 1956). It is proposed that during the co-contraction phase the flight system receives excitatory input but is prevented from becoming active by inhibitory input from tarsal afferents. If co-contraction leads to flight initiation, the time this occurs will depend on the exact balance between these excitatory and inhibitory inputs. Presumably, this balance is such that it limits the earliest time for flight initiation to a point close to the end of co-contraction. Following the triggering of a jump, the flight system is further excited by removal of tarsal inhibition and by excitation from wind-sensitive afferents via interneurons descending from the head. Flight itself will continue to activate the wind-sensitive afferents and hence sustain continuous flight activity.

From a functional viewpoint, the direct linking of jumping to flight is not surprising. Clearly it would be to the animal's advantage to commence flight as soon after the triggering of a jump as possible, and thus have flight initiation independent of the possible variations in afferent input. For example, there may be no significant stimulation of head-hair receptors if the animal jumped in the same direction as the wind. Less obvious is why coupling of flight to jumping should depend on excitatory input to the flight system during the co-contraction phase rather than depending on an input coupled to the triggering of the jump. Wing opening in intact animals normally commences after the jump is triggered (Camhi 1969). Therefore, to have the flight system excited during co-contraction seems inappropriate, particularly

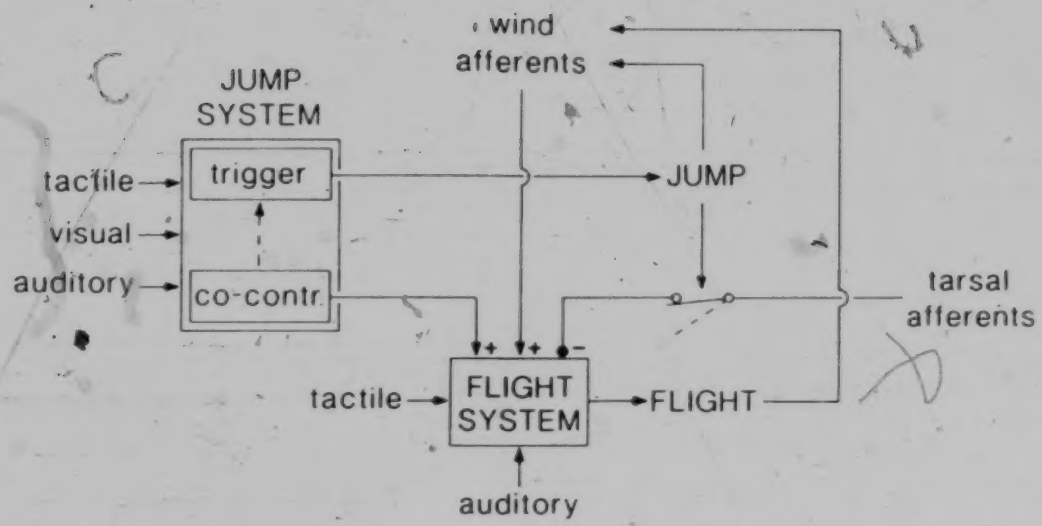

Fig. 6.9. Schematic diagram summarizing the main features of the coupling between the 'jump system' and the 'flight system'. Jumping can be initiated by a variety of stimuli (visual, auditory, tactile) and during the co-contraction phase there is an excitatory input to the flight system. The generation of flight activity in the early part of the co-contraction phase is prevented by an inhibitory influence from tarsal afferents when the animal is in contact with the ground. The time of initiation of flight activity varies depending on the balance of excitatory and inhibitory input to the flight system. In some cases, flight activity can be initiated before the jump is triggered. Following the triggering of a jump, the tarsal inhibition is removed (represented by the open position of the switch) and wind-sensitive afferents on the head are stimulated. Both these effects lead to further excitation of the flight system. Flight itself continues to stimulate wind-sensitive head hairs which then helps maintain activity in the flight system.

because there is considerable variation in the durations and intensities of the co-contraction phases and because not all co-contraction sequences lead to jumps. It can only be speculated that excitation of the flight system during co-contraction facilitates the initiation of wing opening to a greater extent than could be achieved by the trigger system acting alone on the flight system.

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VII. SUMMARY AND CONCLUSIONS

A. ORGANIZATION OF THE JUMP SYSTEM

In this section, a revised scheme of the neuronal mechanisms for jumping and kicking is described (Fig. 7.1). This new scheme takes into consideration the results of the present series of investigations and those of earlier studies. Despite advances made in the understanding of the role of single interneurons (in particular, the M-neurons) in the jump system, the findings of this study still do not allow definitive conclusions to be drawn concerning the system's organization because details of the underlying circuitry are lacking. Consequently, much of what follows is speculative, and the scheme presented here should be viewed as being a tentative one. To describe how this system functions, the mechanisms by which each phase of the motor program, in turn, is generated will be considered.

There are multiple pathways for producing the tibial flexion which initiates the entire motor program, but at present information concerning only one of these pathways is available. Sudden visual or auditory stimuli can activate the mesothoracic C-neurons (not shown in Fig. 7.1) which elicit a specific tibial "cocking" response by monosynaptically exciting hindleg flexor and extensor motoneurons (Pearson and Robertson 1981). However, the cocking response is only produced under the special circumstances in which the locust is highly aroused and its hind tibiae are already close to full flexion. At other times, input from various sensory modalities can still produce the initial flexion phase via a number of undetermined pathways which produce excitation of the flexors alone. In addition, initiation of the motor program must be subject to some form of volitional control because locusts not only jump in response to extrinsic stimuli, but also as a means of locomotion. In these cases, tibial flexion must be produced via a descending command from the brain. The existence of these unknown pathways is denoted by the dashed lines in Fig. 7.1.

Once the tibiae reach the fully flexed position, it is proposed that feedback from tonic units in the femoral CO contributes to the initiation of the co-contraction phase (Chapter 5).

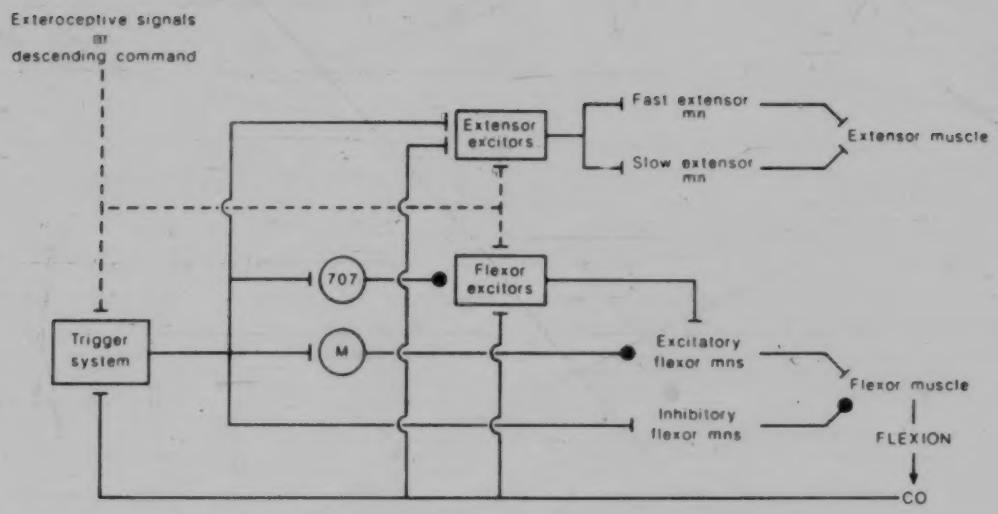

Fig. 7.1. Neuronal organization of the jump system. Only the main features of the system are illustrated. Excitatory connections are indicated by the 'T' bars; filled circles represent inhibitory connections. According to this proposed scheme, once the initial flexion phase has commenced, tonic feedback from the femoral CO (signalling the leg's fully flexed position) makes an important contribution to the generation of the co-contraction phase. This is shown here as excitatory input to the flexor and extensor excitors. Chordotonal input also directly or indirectly activates the central trigger system. Triggering is achieved by the combined effects of direct flexor inhibition (via M), inhibition of the flexor excitors (via 707) and excitation of the inhibitory flexor motoneurons. See text for further details.

This feedback, signalling the angle of femoral-tibial joint, is at least partly responsible for excitating the fast extensor motoneurons and probably also drives the flexor motoneurons during the motor program. Flexor motoneurons often display a sharp transition from low to high frequency spike activity corresponding precisely to the time the first FETi spike is initiated at the onset of co-contraction (e.g. see Figs. 3.2, 5.2, 5.3). This sudden increase in discharge rate can still occur after all extensor activity has been abolished and so is not the result of the central excitatory connection from the fast extensor motoneuron or sensory feedback related to extensor tension (Heitler and Bräunig 1988). It therefore probably results from the same source that produces excitation of the extensors. However, if chordotonal input does provide drive to both flexors and extensors during co-contraction, it probably does so by exciting separate pre-motor systems (as indicated in Fig. 7.1) because flexor and extensor motoneurons do not show common synaptic potentials or similar profiles of synaptic input during the motor program (compare Figs. 2.2 and 2.3; see also Heitler and Burrows 1977a). The central FETi-flexor connection (Hoyle and Burrows 1973) and the numerous positive feedback circuits related to FETi activity (Heitler and Burrows 1977b) cannot be essential components of the system and probably serve only to augment the motor program (Heitler and Bräunig 1988). For this reason, they are not included in Fig. 7.1.

It is important to note that chordotonal input is not solely responsible for generating the co-contraction phase because experimentally inducing flexor and extensor activity when the tibia is flexed does not produce a prolonged co-contraction in the quiescent animal (Heitler and Burrows 1977b). Thus, Heitler and Burrows suggest that a central excitation of the neurons involved in producing co-contraction is also essential for this phase of the motor program to occur. Presumably, this excitation of the interneurons which drive the flexors and extensors then enables the feedback from the CO to initiate co-contraction. The pathways represented by the dashed lines in Fig. 7.1 are intended to include these central excitatory inputs to the jump system.

The trigger phase of the kick or jump program is produced by a system of interneurons whose activation also requires tonic, flexion-dependent feedback from the CO. As described in Chapter 5, the trigger system may either integrate this chordotonal feedback during co-contraction itself or it may integrate excitatory input from the interneurons which excite the flexors and extensors to produce co-contraction. In Fig. 7.1, the first of these possible situations is represented. The trigger system causes the sudden relaxation of the flexor muscles via at least three parallel pathways (Chapter 3). Powerful excitation of the M-neurons ensures the rapid inhibition of all the excitatory flexor motoneurons. Excitation of the 707 interneurons also indirectly terminates activity of the excitatory flexor motoneurons by inhibiting the interneurons which provide flexor drive during co-contraction. The trigger system probably also excites the inhibitory flexor motoneurons to directly reduce tension in the flexor muscle. Since several other interneurons are known to discharge strongly during the trigger phase (Chapter 2), it is likely that additional neurons will be found to play a role in terminating flexor activity.

It now seems probable that the central trigger system is also responsible for producing the burst of extensor activity which commonly occurs at the end of the motor program. In Chapter 2, this possibility was discounted on the basis of the timing of spikes in FETi relative to those in the M-neuron. However, on the few occasions on which the extensor burst preceded the discharge in the M-neuron (e.g. Fig. 2.4B) its timing did correspond to the ramp depolarization in M and may still, therefore, be a result of trigger system input. It is curious, though, why a terminal extensor burst is not seen during every kick. Perhaps the strength of the input from the trigger system is not always sufficient to produce a noticeable increase in discharge frequency in the FETi motoneurons.

One aspect of the pattern of activity in the M- and 707 neurons not accounted for by the scheme in Fig. 7.1 is the hyperpolarization of these cells during the motor program. Since this hyperpolarization commences during the initial flexion phase, it may be produced by those interneurons which provide excitation to the flexors. This inhibitory input to M and 707

would then be switched off during the trigger phase as the 707 interneurons become powerfully activated by the central trigger system. Thus, the jump circuit may contain at least one element of reciprocal inhibition.

The generation of trigger activity does not depend on feedback from the CO alone because periods of co-contraction of the flexor and extensor muscles in quiescent animals can be produced artificially by electrical stimulation and yet trigger activity never ensues (Heitler and Burrows 1977b). Thus, as was true for co-contraction, some central excitatory drive must be required for triggering to occur. This may provide a subthreshold input to the central trigger system, thereby enabling this system to be activated by the feedback from the CO. The level of this central excitation is probably responsible for determining whether a jump or kick will be produced once co-contraction is underway. Episodes of flexor and extensor co-contraction sometimes occur in which the intensity of motor activity gradually declines and no kick or jump results. The proprioceptive feedback at the onset of such "abortive" programs would be normal and so the absence of sustained co-contraction leading to trigger activity is presumably a reflection of decreased levels of central excitation. This gating role of the central excitatory inputs to the trigger system also provides a means for controlling the duration of the motor program. If levels of central excitation were high, the trigger system would be closer to its activation threshold and so trigger activity could be produced after a much shorter period of integration of chordotonal input. When a locust is confronted by a threatening stimulus, the resulting increase in central excitation would then enable it to perform an escape jump or a defensive kick (whichever happened to be appropriate) after a minimal delay. This would explain why in dissected animals that are reluctant to kick (i.e. animals with low levels of central excitation) the periods of co-contraction can sometimes be twice their normal duration. It would also account for the very brief periods of co-contraction displayed by intact, aroused animals (Chapter 2). Extensor activity in these locusts is also very intense - a probable result of central excitation of the co-contraction-producing interneuronal systems.

One of the main conclusions of this investigation is that proprioceptive feedback from the chordotonal organ has at least two important functions in patterning motor activity for jumping and kicking. It is involved in initiating and maintaining the co-contraction phase and is also responsible for terminating co-contraction by generating the trigger phase. Although jumping entails a highly specialized use of the hindlegs, these two roles of proprioceptive input - maintenance of activity during one phase and production of the switch to another phase - have also been found to be important in a variety of animals for patterning the motor activity for which legs are more commonly used, namely walking. In the cockroach, for example, positive feedback from cuticular stress receptors helps to maintain activity in the extensor muscles during the stance phase (Pearson 1972, 1981). A decline in this feedback below a particular level enables the switch to the swing phase to occur. In the stick insect, the femoral CO provides excitatory input to reinforce ongoing activity of the flexor motoneurons as the foreleg flexes during the stance phase. When a certain angle of flexion is reached, however, signals from the CO initiate the swing phase (Bässler 1988). A similar situation exists during the power stroke of the crayfish walking leg where remotor neurons are driven by feedback from a single sensory cell in the muscle receptor organ. A second cell, sensitive to a different aspect of stretch of the receptor organ's muscle, triggers leg promotion to initiate the return stroke (Sillar et al. 1986). The unique aspect of the locust jump system, though, is that the sensory feedback that is involved in maintaining one phase of the program is also responsible for causing the switch to the subsequent phase. This is because the switch is not simply produced by a phasic afference signalling that a certain state has been reached, but rather by the central integration of the same tonic feedback which maintained motor activity during the program's preceding phase.

Another aspect not included in the scheme illustrated in Fig. 7.1 is the linkage between the jump system and the system which produces flight. As shown in Chapter 6, these two behaviors are centrally coupled, but this does not appear to be a result of phasic excitation from the trigger system. Instead, the flight system receives excitatory input throughout the

co-contraction phase. One possible explanation for this is that the chordotonal feedback which is responsible for generating co-contraction also provides a facilitatory input to the flight system. Another possibility is that the circuitry underlying these two behaviors is intimately linked, such that the neurons providing excitation to the flexors and extensors during co-contraction also provide the facilitatory input to interneurons of the flight system. In both cases, the coupling of flight initiation to the jump would ultimately be due to sensory feedback from the chordotonal organ. Thus, not only are chordotonal afferences important for patterning motor activity during the jump, they may also play a key role in initiating motor activity for an entirely different mode of locomotion.

The previous model of the neuronal mechanisms for jumping proposed that triggering was produced by a combination of exteroceptive and extensor-related proprioceptive inputs activating a single pair of interneurons (Pearson et al. 1980; Steeves and Pearson 1982). Clearly, this picture has now been radically altered. Extrinsic sensory stimuli may serve to initiate the program but do not influence subsequent aspects of its performance (Chapter 4). Contingent on the existence of CO input, the co-contraction and trigger phases are produced by a program whose time course is determined entirely by central mechanisms. Flexor activity and the resultant flexion-related proprioceptive feedback exert primary control over the motor program's production, and the main function of extensors in this system is to generate the high levels of force required to power the leg's extension during the kick or jump.

B. FUTURE WORK

Although this series of investigations has shed new light on the neuronal mechanisms underlying jumping and kicking, the present understanding of how this system functions is far from complete. One area in particular in which knowledge is lacking concerns the integration of proprioceptive information during the motor program. Given the success of the approach used by Burrows and his colleagues in determining the mechanisms by which various sensory inputs from the legs are processed (e.g. Siegler and Burrows 1983, 1986; Burrows and Siegler

1985; Burrows and Pflüger 1986; Laurent 1986, 1987a,b), an analysis of the integration of proprioceptive information during kicking should be quite feasible. Indeed, discrete populations of spiking local interneurons have already been found to be important primary integrators of afferent input from the hindleg femoral CO (Burrows 1987). However, the great majority of these neurons are probably involved in processing information in reflex pathways and consequently may be of little importance for production of the kick or jump motor program. The focus of future studies, then, should be to determine which spiking local interneurons receive synaptic input from the small number of chordotonal units which are responsive to femoral-tibial angles close to full flexion, as these are likely to be the ones critical for performance of jumps. Zill (1985) has shown that intracellular recordings can be made from single units in the CO (or from their afferent fibres) and so an identification of the important local interneurons should be possible. Once this has been achieved, the next step would be to record the pattern of activity in these neurons during kicks and to determine details of their connectivity with other interneurons. Burrows (1987) suggests that the spiking local neurons which receive input from chordotonal afferents very likely distribute their coded information to nonspiking and intersegmental interneurons. This course of investigation, then, might lead naturally to the discovery of interneurons belonging to the central trigger system. At present, the identities of the neurons which comprise the trigger system have not been determined and consequently nothing is known about how the system is organized or how it functions to generate the trigger signal.

Another approach that could be adopted to identify member neurons of the central trigger system would be to attack the system from its output side. Presumably, any neuron which was found to provide strong excitatory input to the M-neurons or to the 707 neurons would almost certainly be an important element of jump circuit. After such neurons have been identified, details of their connectivity could be determined. In addition, perturbation of the activity of these interneurons during the motor program should produce "resetting" effects similar to those obtained by chordotonal afferent stimulation and thereby provide further

clues as to the functioning of the trigger system.

The strategy described above for examining the central integration of proprioceptive input will very likely yield new insights into another aspect of the jump circuitry about which there is currently very little information, namely the mechanisms for producing co-contraction. It should resolve the question of whether the neuronal systems for generating the co-contraction and trigger phases of the motor program are indeed coupled or whether they simply share a common dependence on chordotonal feedback for their activation. Moreover, knowledge of the interneurons which are responsible for exciting the flexor and extensor motoneurons may also provide a solution to the problem of the origin of the facilitatory input to the flight system during the co-contraction phase, thereby revealing the mechanisms by which jumping and flying behaviors are linked.

Clearly, much remains to be determined about the cellular basis for kicking and jumping in the locust. However, given the relative ease with which microelectrode recordings can be made from these animals whilst kicks are performed, the jumping system of the locust appears to offer an excellent preparation in which to further our understanding of the way sensory inputs are processed by the central nervous system in generating a complex pattern of motor output.

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